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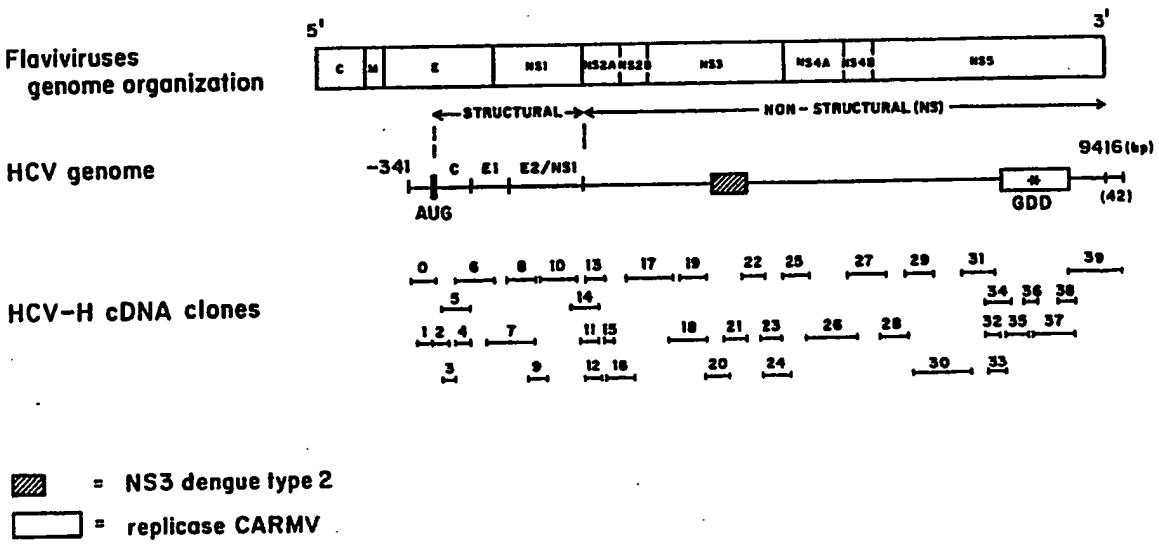

(57) Abstract

The present invention relates to a DNA segment encoding a recombinant non-A, non-B hepatitis structural protein or fusion protein and a recombinant DNA (rDNA) molecule capable of expressing either protein. Cells transformed with the rDNA, methods for producing the proteins in addition to compositions containing the proteins, and their use in diagnostic methods and systems, and in vaccines are also described.

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NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

Description

Technical Field

The present invention relates to a segment of deoxyribonucleic acid (DNA) that encodes a non-A, non-B hepatitis structural protein and a recombinant DNA (rDNA) that contains the DNA segment. Cells transformed with a rDNA of the present invention and methods for producing the NANBV structural protein are also contemplated. The invention also describes compositions containing a NANBV structural protein useful in diagnostic methods and in vaccines.

Background of the Invention

Non-A, non-B hepatitis (NANBH) is believed to be caused by a transmissible virus that has been referred to as both hepatitis C virus (HCV) and non-A, non-B hepatitis virus (NANBV). Although the transmissible disease was discovered years ago, a complete characterization of the causative agent is still being developed.

Isolates of NANBV have been obtained and portions or all of the viral genome of the various isolates were molecularly cloned and sequenced. Choo et al, Science, 244:359-362 (1989); Choo et al., Proc. Natl. Acad. Sci. USA, 88:2451-2455 (1991); Takamizawa et al., J. Virol., 65:1105-1113 (1991); Kato et al., Proc. Natl. Acad. Sci. USA, 87:9524-9528 (1990); and Takeuchi et al., Nucl. Acids Res., 18:4626 (1990). Similarities in nucleotide base sequence between the different isolates of NANBV suggest that they are a part of a family of related viruses. Okamoto et al, Japan J. Exp. Med., 60:163-177 (1990); and Ogata et al., Proc. Natl. Acad. Sci. USA, 88:3392-3396 (1991).

Properties of the NANBV genome suggest that NANBV may be a very distant relative of the flavivirus family. However, similarities in both the size and hydropathicity of the structural proteins suggest that NANB viruses may also be distantly related to the pestivirus family. Miller et al., Proc. Natl. Acad. Sci., 87:2057-2061 (1990); and Okamoto et al., Japan J. Exp. Med., 60:163-177 (1990).

The difficulties in characterizing the NANBV isolates taxonomically and the lack of information regarding the proteins encoded by the NANBV genome have made it difficult to identify relevant gene products useful for diagnostic markers and for producing NANBV vaccines.

The NANBV genome is comprised of a positive stranded RNA molecule that codes for a single polyprotein. The gene products of NANBV are believed to include both structural and nonstructural proteins, based on homologies to characterized, related viruses. From these homologies, it is predicted that NANBV expresses a single polyprotein gene product from the complete viral genome, which is then cleaved into functionally distinct structural and nonstructural proteins. This type of viral morphogenesis precludes positive identification of the individual mature viral proteins until they have been physically isolated and characterized. Since no in vitro culturing system to propagate the virus has been developed for NANBV, no NANBV structural or nonstructural gene products (proteins) have been isolated from biological specimens or NANBV-infected cells. Thus, the identification of NANBV proteins, of their role in the viral life cycle, and of their role in disease, have yet to be determined. In particular, antigenic markers

for NANBV-induced disease have yet to be fully characterized.

One NANBV gene product, namely the antigen C100-3, derived from portions of the nonstructural genes designated NS3 and NS4, has been expressed as a fusion protein and used to detect anti-C100-3 antibodies in patients with various forms of NANB hepatitis. See, for example, Kuo et al, Science, 244:362-364 (1989); and International Application No. PCT/US88/04125. A diagnostic assay based on C100-3 antigen is commercially available from Ortho Diagnostics, Inc. (Raritan, NJ). This C100-3 assay currently represents the state of the art in detecting NANBV infections. However, the C100-3 antigen-based immunoassay has been reported to preferentially detect antibodies in sera from chronically infected patients. C100-3 seroconversion generally occurs from four to six months after the onset of hepatitis, and in some cases C100-3 fails to detect any antibody where an NANBV infection is present. Alter et al., New Eng. J. Med., 321:1538-39 (1989); Alter et al., New Eng. J. Med., 321:1494-1500 (1989); and Weiner et al., Lancet, 335:1-3 (1990). McFarlane et al., Lancet, 335:754-757 (1990), described false positive results when the C100-3-based immunoassay was used to measure antibodies in patients with autoimmune chronic active hepatitis. Using the C100-3-based immunoassay, Grey et al., Lancet, 335:609-610 (1990), describe false positive results on sera from patients with liver disease caused by a variety of conditions other than NANBV.

A NANBV immunoassay that could accurately detect seroconversion at early times after infection, or that could identify an acute NANBV infection, is not presently available.

The Hutch strain of HCV is a clinically interesting isolate compared to the Donn strain (HCV-1) because HCV-H grows to extremely high titers in the patient.

Summary of the Invention

One Hutch strain (HCV-H) of non-A, non-B hepatitis virus (NANBV) designated the Hutch c59 isolate (or HCV-Hc59) has been propagated through passage in animals and the entire viral genome has been cloned and sequenced. When using the term "subgroup" the present specification refers to a group of NANBVs which is serologically defined by particular strains, such as the Hutch c59 strain. Sequence data shows differences at both the nucleotide and amino acid level when compared to previously reported NANBV strains. See, the sequences of the following HCV isolates, where the isolate designation is shown in parenthesis for comparison, Okamoto et al, Japan J. Exp. Med., 60:163-177, 1990 (HC-J1, HC-J4); Takeuchi et al., Nucleic Acids Res., 18:4626, 1990 (HCV-JH); Choo et al., Proc. Natl. Acad. Sci. USA, 88:2451-2455, 1991 (HCV-1); Kato et al., Proc. Natl. Acad. Sci. USA, 87:9524-9528, 1990 (HCV-J); Takamizawa et al., J. Virol., 65:1105-1113, 1991 (HCV-BK); United States Patent No. 5,032,511 to Takahashi et al.; Ogata et al., Proc. Natl. Acad. Sci. USA, 88:3392-3396, 1991 (HCV-Hh); and International Application No. PCT/US88/04125.

The identified sequences have been shown herein to encode structural proteins of NANBV. The NANBV structural proteins are also shown herein to include antigenic epitopes useful for diagnosis of antibodies immunoreactive with structural proteins of NANBV, and for use in vaccines to induce neutralizing antibodies

against NANBV. In particular, the NANBV antigens of this invention are Hutch c59 isolate NANBV antigens.

The nucleotide sequence that codes for the amino terminal polyprotein portion of the structural genes of the Hutch strain of NANBV is contained in SEQ ID NO:1. By comparison to other NANBV isolates, to flavivirus, and to pestivirus, the nucleotide sequence contained in SEQ ID NO:1 is believed to encode structural proteins of NANBV, namely capsid and portions of envelope.

The structural antigens described herein are present in the putative capsid protein contained in SEQ ID NO:1 from amino acid residue positions 1-120, and are present in the amino terminal portion of the putative envelope protein contained in SEQ ID NO:1 from residue positions 121 to 326.

Nucleotide and amino acid residue sequences are defined herein from a starting base or amino acid residue position number to an end base or residue position number. It is understood that all such sequences include both the starting and end position numbers.

The complete sequence of the genome of the Hutch c59 isolate has also been determined and is described. Thus, the present invention contemplates a DNA segment encoding the viral genome of the Hutch c59 isolate of NANBV contained in SEQ ID NO:46 from nucleotide position 1 to 9416.

The present invention also contemplates a DNA segment encoding a NANBV structural protein that comprises a NANBV structural antigen, preferably capsid antigen. A particularly preferred capsid antigen includes an amino acid residue sequence represented by SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to

residue 40, or from residue 1 to residue 74, and the DNA segment preferably includes the nucleotide base sequence represented by SEQ ID NO:1 from base position 1 to base position 60, from base position 61 to base position 120, from base position 4 to base position 120, or from base position 1 to base position 222, respectively.

A polynucleotide is also contemplated comprising a nucleotide sequence that encodes portions of the Hutch c59 isolate polyprotein, particularly portions of the sequence-specific regions of c59 in the V, V_1, V_2 or V_3 region.

Also contemplated is a recombinant DNA molecule comprising a vector, preferably an expression vector, operatively linked to a DNA segment of the present invention. A preferred recombinant DNA molecule is pGEX-3X-690:691, pGEX-3X-690:694, pGEX-3X-693:691, PGEX-3X-15:17, pGEX-3X-15:18, pGEX-2T-15:17, pGEX-2T-CAP-A, pGEX-2T-CAP-B or pGEX-2T-CAP-A-B.

A NANBV structural protein is contemplated that comprises an amino acid residue sequence that defines a NANBV structural antigen, preferably a capsid antigen, and more preferably one that includes the amino acid residue sequence contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, or from residue 1 to residue 74. Fusion proteins comprising a NANBV structural protein of this invention are also contemplated.

The invention also contemplates an antibody containing antibody molecules that immunoreact with the Hutch c59 isolate of NANBV, but do not immunoreact with NANBV isolates HCV-1, HCV-BK, HCV-J, HC-J1, HC-J4, HCV-JH or HCV-Hh, i.e., c59-specific antibody molecules.

Further contemplated is a culture of cells transformed with a recombinant DNA molecule of this invention and methods of producing a NANBV structural protein of this invention using the culture.

Also contemplated is a composition comprising a NANBV structural protein. The composition is preferably characterized as being essentially free of (a) procaryotic antigens, and (b) other NANBV-related proteins.

Still further contemplated is a diagnostic system in kit form comprising, in an amount sufficient to perform at least one assay, a NANBV structural protein composition, a polypeptide or a fusion protein of this invention, as a separately packaged reagent. Preferably, the diagnostic system contains the fusion protein affixed to a solid matrix.

Further contemplated is a method, preferably an in vitro method, of assaying a body fluid sample for the presence of antibodies against at least one of the NANBV structural antigens described herein. The method comprises forming an immunoreaction admixture by admixing (contacting) the body fluid sample with an immunological reagent such as a NANBV structural protein, polypeptide or fusion protein of this invention. The immunoreaction admixture is maintained for a time period sufficient for any of the antibodies present to immunoreact with the admixed immunological reagent to form an immunoreaction product, which product, when detected, is indicative of the presence of anti-NANBV structural protein antibodies. Preferably, the immunological reagent is affixed to a solid matrix when practicing the method.

The invention also contemplates a method, preferably an in vitro method, of assaying a body sample for the presence of NANBV polynucleic acids.

The method generally comprises a) forming an aqueous hybridization admixture by admixing a body sample with an polynucleotide of this invention; b) maintaining the aqueous hybridization admixture for a time period and under hybridizing conditions sufficient for any NANBV polynucleic acids present in the body sample to hybridize with the admixed polynucleotides to form a hybridization product; and c) detecting the presence of any of the hybridization product formed and thereby the presence of NANBV polynucleic acids in the body sample.

In another embodiment, this invention contemplates an inoculum (or a vaccine) comprising an immunologically effective amount of a NANBV structural protein, polypeptide or fusion protein of this invention dispersed in a pharmaceutically acceptable carrier and/or diluent. The inoculum is essentially free of (a) procaryotic antigens, and (b) other NANBV-related proteins.

A prophylactic method for treating infection, which method comprises administering an inoculum of the present invention, is also contemplated.

Brief Description of the Drawings

Figure 1 is a schematic representation of the HCv-Hc59 genome and location of HCV-Hc59 cDNA clones numbered from zero to 39. Alignment with the protein encoded by flaviviruses is shown as well as the putative domains in the HCV encoded genome. Regions of amino acid homology with the Dengue Type 2 Ns3 virus and the Carnation Mottle virus (CARMv) are indicated by striped and empty boxes, respectively.

Detailed Description of the Invention

A. Definitions

Amino Acid: All amino acid residues identified herein are in the natural L-configuration. In keeping with standard polypeptide nomenclature, J. Biol. Chem., 243:3557-59, (1969), abbreviations for amino acid residues are as shown in the following Table of Correspondence:

TABLE OF CORRESPONDENCE

SYMBOL 3-Letter	AMINO ACID
Tyr	L-tyrosine
Gly	L-glycine
Phe	L-phenylalanine
Met	L-methionine
Ala	L-alanine
Ser	L-serine
Ile	L-isoleucine
Leu	L-leucine
Thr	L-threonine
Val	L-valine
Pro	L-proline
Lys	L-lysine
His	L-histidine
Gln	L-glutamine
Glu	L-glutamic acid
Glx	Gln or Glu
Trp	L-tryptophan
Arg	L-arginine
Asp	L-aspartic acid
Asn	L-asparagine
Asx	Asp or Asn
Cys	L-cysteine
Xaa	Unknown or other

It should be noted that all amino acid residue sequences, typically referred to herein as "residue sequences", are represented herein by formulae whose left to right orientation is in the conventional direction of amino-terminus to carboxy-terminus.

Antigen: A polypeptide or protein that is able to specifically bind to (immunoreact with) an antibody and form an immunoreaction product (immunocomplex). The site on the antigen with which the antibody binds is referred to as an antigenic determinant or epitope.

Nucleotide: a monomeric unit of DNA or RNA consisting of a sugar moiety (pentose), a phosphate, and a nitrogenous heterocyclic base. The base is linked to the sugar moiety via the glycosidic carbon (1' carbon of the pentose) and that combination of base and sugar is a nucleoside. When the nucleoside contains a phosphate group bonded to the 3' or 5' position of the pentose it is referred to as a nucleotide. A sequence of operatively linked nucleotides is typically referred to herein as a "base sequence", and is represented herein by a formula whose left to right orientation is in the conventional direction of 5'-terminus to 3'-terminus.

Duplex DNA: A double-stranded nucleic acid molecule comprising two strands of substantially complementary polynucleotide hybridized together by the formation of a hydrogen bond between each of the complementary nucleotides present in a base pair of the duplex. Because the nucleotides that form a base pair can be either a ribonucleotide base or a deoxyribonucleotide base, the phrase "duplex DNA" refers to either a DNA-DNA duplex comprising two DNA strands (ds DNA), or an RNA-DNA duplex comprising one DNA and one RNA strand.

Base Pair (bp): a partnership of adenine (A) with thymine (T), or of cytosine (C) with guanine (G) in a double stranded DNA molecule. In RNA, uracil (U) is substituted for thymine.

Complementary Nucleotide Sequence: a sequence of nucleotides in a single-stranded molecule of DNA or RNA that is sufficiently complementary to that on another single strand to specifically (non-randomly) hybridize to it with consequent hydrogen bonding.

Hybridization: the pairing of complementary nucleotide sequences (strands of nucleic acid) to form a duplex, heteroduplex or complex containing more than two single-stranded nucleic acids by the establishment of hydrogen bonds between/among complementary base pairs. It is a specific, i.e. non-random, interaction between/among complementary polynucleotides that can be competitively inhibited.

Hybridization Product: The product formed when a polynucleotide hybridizes to a single or double-stranded nucleic acid. When a polynucleotide hybridizes to a double-stranded nucleic acid, the hybridization product formed is referred to as a triple helix or triple-stranded nucleic acid molecule. Moser et al, Science, 238:645-50 (1987).

Nucleotide Analog: a purine or pyrimidine nucleotide that differs structurally from a A, T, G, C, or U, but is sufficiently similar to substitute for the normal nucleotide in a nucleic acid molecule. Inosine (I) is a nucleotide that can hydrogen bond with any of the other nucleotides, A, T, G, C, or U. In addition, methylated bases are known that can participate in nucleic acid hybridization.

B. DNA Segments

In living organisms, the amino acid residue sequence of a protein or polypeptide is directly

related via the genetic code to the deoxyribonucleic acid (DNA) sequence of the structural gene that codes for the protein. Thus, a structural gene can be defined in terms of the amino acid residue sequence, i.e., protein or polypeptide, for which it codes.

An important and well known feature of the genetic code is its redundancy. That is, for most of the amino acids used to make proteins, more than one coding nucleotide triplet (codon) can code for or designate a particular amino acid residue. Therefore, a number of different nucleotide sequences may code for a particular amino acid residue sequence. Such nucleotide sequences are considered functionally equivalent since they can result in the production of the same amino acid residue sequence in all organisms. Occasionally, a methylated variant of a purine or pyrimidine may be incorporated into a given nucleotide sequence. However, such methylations do not affect the coding relationship in any way.

In one embodiment the present invention contemplates an isolated DNA segment that comprises a nucleotide base sequence that encodes a NANBV structural protein comprising a NANBV structural antigen such as a capsid antigen, an envelope antigen, or both. Preferably, the structural antigen is immunologically related to the Hutch strain of NANBV.

More preferably, the encoded NANBV structural antigen has an amino acid residue sequence that corresponds, and preferably is identical, to the amino acid residue sequence contained in SEQ ID NO:1.

In one embodiment, the putative capsid antigen includes an amino acid residue sequence contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, or from residue 1 to residue 74. In another embodiment,

the capsid antigen includes the sequence contained in SEQ ID NO:1 from residue 69 to residue 120.

In another embodiment, the putative envelope antigen includes an amino acid residue sequence contained in SEQ ID NO:1 from residue 121 to residue 176 or from residue 121 to residue 326.

Preferred DNA segments include a base sequence represented by the base sequence contained in SEQ ID NO:1 from base position 1 to base position 222, from base position 205 to base position 360, from base position 361 to base position 528, or from base position 361 to base position 978.

In preferred embodiments, the length of the nucleotide base sequence is no more than about 3,000 bases, preferably no more than about 1,000 bases.

The amino acid residue sequence of a particularly preferred NANBV structural protein is contained in SEQ ID NO:2 from residue 1 to residue 315, in SEQ ID NO:3 from residue 1 to residue 252, in SEQ ID NO:4 from residue 1 to residue 252 and in SEQ ID NO:6 from residue 1 to residue 271.

A purified DNA segment of this invention is substantially free of other nucleic acids that do not contain the nucleotide base sequences specified herein for a DNA segment of this invention, whether the DNA segment is present in the form of a composition containing the purified DNA segment, or as a solution suspension or particulate formulation. By substantially free is meant that the DNA segment is present as at least 10% of the total nucleic acid present by weight, preferably greater than 50 percent, and more preferably greater than 90 percent of the total nucleic acid by weight.

In another embodiment, a DNA segment of this invention contains a nucleotide base sequence that

defines a structural gene capable of expressing a fusion protein. The phrase "fusion protein" refers to a protein having a polypeptide portion operatively linked by a peptide bond to a second polypeptide portion defining a NANBV structural antigen as disclosed herein.

A preferred first polypeptide portion has an amino acid residue sequence corresponding to a sequence as contained in SEQ ID NO:2 from about residue 1 to about residue 221, and is derived from the protein glutathione-S-transferase (GST).

A preferred second polypeptide portion defining a NANBV structural antigen in a fusion protein includes an amino acid residue sequence represented by the sequence contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, from residue 1 to residue 74, from residue 69 to residue 120, from residue 121 to residue 176, or from residue 121 to residue 326.

In one embodiment, a fusion protein can contain more than one polypeptide portion defining a NANBV structural antigen, as for example the combination of two polypeptide portions representing different structural antigens as shown by the amino acid residue sequence contained in SEQ ID NO:1 from residue 1 to residue 120, or in SEQ ID NO:1 from residue 1 to residue 326.

In particularly preferred embodiments, that portion of a fusion protein encoding DNA segment of this invention that codes for the polypeptide portion defining a NANBV capsid antigen includes a nucleotide base sequence corresponding to a sequence that codes for an amino acid residue sequence as contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, or from

residue 1 to residue 74, and more preferably includes a nucleotide base sequence corresponding to a base sequence as contained in SEQ ID NO:1 from base 1 to base 60, from base 61 to base 120, from base 4 to base 120, or from base 1 to base 222, respectively.

In another embodiment, that portion of a fusion protein encoding DNA segment of this invention that codes for the polypeptide portion defining a NANBV envelope antigen includes a nucleotide base sequence corresponding to a sequence that codes for an amino acid residue sequence as contained in SEQ ID NO:1 from residue 121 to residue 176 or from residue 121 to residue 326, and more preferably includes a nucleotide base segment corresponding in base sequence to the nucleotide base sequence contained in SEQ ID NO:1 from base 361 to base 528 or from base 361 to base 978, respectively.

A particularly preferred fusion protein encoding DNA segment of this invention has a nucleotide base sequence corresponding to the sequence contained in SEQ ID NO:2 from base 1 to base 945, SEQ ID NO:3 from base 1 to base 756, SEQ ID NO:4 from base 1 to base 756, and SEQ ID NO:6 from base 1 to base 813.

In preferred embodiments, a DNA segment of the present invention is bound to a complementary DNA segment, thereby forming a double stranded DNA segment. In addition, it should be noted that a double stranded DNA segment of this invention preferably has a single stranded cohesive tail at one or both of its termini.

In another embodiment, a DNA segment of the present invention comprises a nucleotide base sequence that encodes the genome of the Hutch isolate of NANBV. Preferably, the DNA segment has a nucleotide base sequence that encodes the amino acid residue sequence

of the polyprotein produced by the genome of the Hutch c59 isolate, which amino acid residue sequence is shown in SEQ ID NO:46 from residue 1 to residue 3011. More preferably, the DNA segment in this embodiment has the nucleotide sequence shown in SEQ ID NO:46 from base 1 to base 9416.

A DNA segment encoding the c59 isolate genome is useful for the preparation of a hybridization standard or control in diagnostic methods based on nucleic acid hybridization using the polynucleotides, for the preparation of NANBV structural antigens or fusion proteins by recombinant DNA methods, for the preparation of infectious NANBV c59 isolate particles in culture, and the like, all of which are described herein.

In another embodiment, the present invention contemplates a fragment of a DNA segment of this invention corresponding to a portion of a NANBV genome or encoding a portion of a NANBV structural antigen. These fragments, when present in single stranded form or specified in the context of one strand of a double stranded DNA segment, are referred to herein as polynucleotides.

Where the polynucleotide is used to encode a NANBV structural antigen, or region of the Hutch c59 isolate polyprotein, the polynucleotide corresponds to the coding strand of a NANBV genome as described herein. Where the polynucleotide is used as a hybridization probe or primer for hybridization with NANBV-derived nucleic acids, the sense of the strand will depend, as is well known upon the target sequence to which hybridization is directed.

Thus in one embodiment, the present invention contemplates a polynucleotide that comprises a nucleotide base sequence that includes a nucleotide

base sequence that encodes an amino acid residue sequence corresponding to a portion of the polyprotein expressed by the Hutch isolate of NANBV. Preferably the polynucleotide encodes a sequence that corresponds to a portion of the amino acid residue sequence of the c59 isolate shown in SEQ ID NO:46 from residue 1 to residue 3011.

Particularly preferred are regions of the Hutch c59 isolate which are unique and thereby provide a means to distinguish the Hutch isolate, and more preferably the c59 isolate, from other isolates of NANBV on the basis of amino acid residue or nucleotide base sequence differences. Regions of the genome of the c59 isolate useful for distinguishing isolates contain differences in nucleotide base sequence, and preferably define differences in the encoded amino acid residue sequence, when compared to the nucleotide or amino acid residue sequence of the isolate to be distinguished.

Representative comparisons to identify Hutch isolate sequence differences are shown herein in the Examples, and particularly in Table 11.

Thus, a polynucleotide of this invention in one embodiment comprises a nucleotide base sequence that includes a nucleotide sequence that encodes an amino acid residue sequence that corresponds to a portion of the sequence of the Hutch c59 isolate of NANBV shown in SEQ ID NO:46 such that the polynucleotide has at least one nucleotide base difference in sequence when compared to the nucleotide sequence of a strain of NANBV selected from the group consisting of HCV-1, HCV-BK, HCV-J, HC-J1, HC-J4, HCV-JH and HCV-Hh. Preferably the nucleotide base sequence includes a sequence defining a portion of the variable region of the NANBV genome selected from the group consisting

of: the V variable region nucleotide base sequence (base 1497 to base 1574 of SEQ ID NO:46), the V_1 variable region nucleotide base sequence (base 1077 to base 1166 of SEQ ID NO:46), the V_2 variable region nucleotide base sequence (base 1707 to base 1787 of SEQ ID NO:46), and the V_3 variable region nucleotide base sequence (base 7407 to base 7478 of SEQ ID NO:46).

The SEQ ID NO and corresponding bases of the sequence are referred to herein conveniently in parenthesis following a reference to a sequence. For example, the sequence of nucleotides from base 1 to base 9416 shown in SEQ ID NO:46 is referred to as "46:1-9416".

Particularly preferred polynucleotides have a nucleotide base sequence selected from the group consisting of the V variable region nucleotide base sequence (46:1497-1574), the V_1 variable region nucleotide base sequence (46:1077-1166), the V_2 variable region nucleotide base sequence (46:1707-1787), and the V_3 variable region nucleotide base sequence (46:7407-7478).

In another embodiment, a polynucleotide comprises a nucleotide base sequence that includes a nucleotide sequence that encodes an amino acid residue sequence selected from the group consisting of residue 391 to residue 404 of SEQ ID NO:46, residue 246 to residue 256 of SEQ ID NO:46, residue 461 to residue 466 of SEQ ID NO:466, residue 473 to residue 482 of SEQ ID NO:46, and residue 2356 to residue 2379 of SEQ ID NO:46. Preferably, the included nucleotide sequence corresponds to the sequence shown in SEQ ID NO:46. The above-indicated ranges of amino acid residues correspond to portions of the V, V_1, V_2 and V_3 regions that contain the greatest amount of sequence diversity

when compared to known HCV isolates, and therefore are most preferred.

For reasons of ease of synthesis and sequence specificity, preferred polynucleotides are from about 10 to about 200 nucleotides in length, although the particular length will depend upon the purpose for using the polynucleotide.

A polynucleotide for use in the present invention in its various embodiments includes a primer, a probe, or a nucleic acid.

The term "probe" as used herein refers to a polynucleotide, whether purified from a nucleic acid restriction digest or produced synthetically, which is about 8 to 200 nucleotides in length, having a nucleotide base sequence that is substantially complementary to a predetermined specific nucleic acid sequence present in a gene of interest, i.e. a target nucleic acid.

The polynucleotide probe must be sufficiently long to be capable of hybridizing under hybridizing conditions with a specific nucleic acid sequence present in the gene of interest. The exact length of the polynucleotide probe will depend on many factors, including hybridization temperature and the nucleotide sequence of the probe. For example, depending on the complexity of the target sequence, a polynucleotide probe typically contains 15 to 25 or more nucleotides, although it can contain fewer nucleotides. As few as 8 nucleotides in a polynucleotide have been reported as effective for use. Studier et al, Proc. Natl. Acad. Sci. USA, 86:6917-21 (1989). Short polynucleotide probes generally require lower temperatures to form sufficiently stable hybrid complexes with target 5.

In preferred embodiments a polynucleotide probe has a size of less than about 200 nucleotides in length, preferably less than 100 nucleotides, and more preferably less than 30 nucleotides.

By "substantially complementary" and its grammatical equivalents in relation to a probe is meant that there is sufficient nucleotide base sequence similarity between a subject polynucleotide probe and a specific nucleic acid sequence present in a gene of interest that the probe is capable of hybridizing with the specific sequence under hybridizing conditions and form a duplex comprised of the probe and the specific sequence.

Therefore, the polynucleotide probe sequence may not reflect the exact sequence of the target sequence so long as the probe contains substantial complementarity with the target sequence. For example, a non-complementary polynucleotide can be attached to the one end of the probe, with the remainder of the probe sequence being substantially complementary to the target sequence. Such non-complementary polynucleotides might code for an endonuclease restriction site or a site for protein binding. Alternatively, non-complementary bases or longer sequences can be interspersed into the probe, provided the probe sequence has sufficient complementarity with the sequence of the target strand as to non-randomly hybridize therewith and thereby form a hybridization product under hybridization conditions.

The polynucleotide probe is provided in single-stranded form for maximum efficiency, but may alternatively be double stranded. If double stranded, the polynucleotide probe is first treated to separate its strands before being used in hybridization to

prepare hybridization products. Preferably, the probe is a polydeoxyribonucleotide.

A DNA segment or polynucleotide of the present invention can easily be prepared from isolated virus obtained from the blood of a NANBV-infected individual such as described herein or can be synthesized de novo by chemical techniques.

De novo chemical synthesis of a DNA segment or a polynucleotide can be conducted using any suitable method, such as, for example, the phosphotriester or phosphodiester methods. See Narang et al., Meth. Enzymol., 68:90, (1979); U.S. Patent No. 4,356,270; Itakura et al., Ann. Rev. Biochem., 53:323-56 (1989); Brown et al., Meth. Enzymol., 68:109, (1979); and Matteucci et al., J. Am. Chem. Soc., 103:3185 (1981). (The disclosures of the art cited herein are incorporated herein by reference.) Of course, by chemically synthesizing the structural gene portion, any desired modifications can be made simply by substituting the appropriate bases for those encoding a native amino acid residue. However, DNA segments including sequences identical to a segment contained in SEQ ID NOS 1, 2, 3, 4 or 6 are preferred.

Derivation of a polynucleotide from nucleic acids involves the cloning of a nucleic acid into an appropriate host by means of a cloning vector, replication of the vector and therefore multiplication of the amount of the cloned nucleic acid, and then the isolation of subfragments of the cloned nucleic acids. For a description of subcloning nucleic acid fragments, see Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, pp 390-401 (1982); and see U.S. Patents No. 4,416,988 and No. 4,403,036.

In addition, a DNA segment can be prepared by first synthesizing oligonucleotides that correspond to portions of the DNA segment, which oligonucleotides are then assembled by hybridization and ligation into a complete DNA segment. Such methods are also well known in the art. See for example, Paterson et al., Cell, 48:441-452 (1987); and Lindley et al., Proc.Natl. Acad. Sci., 85:9199-9203 (1988), where a recombinant peptide, neutrophil-activated factor, was produced from the expression of a chemically synthesized gene in E. coli.

A DNA segment of this invention can be used for the preparation of rDNA molecules, in the construction of vectors for expressing a NANBV structural protein or fusion protein of this invention, or as a hybridization probe for detecting the presence of NANBV specific nucleic acid sequences in samples.

Where the use of a DNA segment is for preparing proteins, the specified amino acid residue is considered important, and the nucleotide base sequence of the DNA segment can vary based on the redundancy of the genetic code, as is well known, to provide for the desired amino acid residue sequence.

Where the use of a DNA segment is as a hybridization probe for specific nucleic acid sequences, it is a nucleotide base sequence corresponding to the Hutch strain NANBV nucleotide base sequences disclosed herein that is preferred.

C. Recombinant DNA Molecules

The present invention further contemplates a recombinant DNA (rDNA) that includes a DNA segment of the present invention operatively linked to a vector. A preferred rDNA of the present invention is characterized as being capable of directly expressing, in a compatible host, a NANBV structural protein or

fusion protein of this invention. Preferred DNA segments for use in a rDNA are those described herein above.

By "directly expressing" is meant that the mature polypeptide chain of the protein is formed by translation alone as opposed to proteolytic cleavage of two or more terminal amino acid residues from a larger translated precursor protein. Preferred rDNAs of the present invention are the plasmids pGEX-3X-690:694 , pGEX-3X-693:691, pGEX-3X-690:691, pGEX-3X-15:17, pGEX-3X-15:18, pGEX-2T-15:17, pGEX-2T-CAP-A, pGEX-2T-CAP-B, and pGEX-2T-CAP-A-B described in Example 1.

A recombinant DNA molecule (rDNA) of the present invention can be produced by operatively linking a vector to a DNA segment of the present invention. Exemplary rDNA molecules and the methods for their preparation are described in Example 1.

In another embodiment, a rDNA molecule of this invention comprises a vector operatively linked to a DNA segment comprising a nucleotide base sequence that encodes the genome of the Hutch isolate of NANBV. Preferably, the rDNA molecule includes a nucleotide base sequence that encodes the amino acid residue sequence of the polyprotein produced by the genome of the Hutch c59 isolate, which amino acid residue sequence is shown in SEQ ID NO:46 from residue 1 to residue 3011. More preferably, the rDNA molecule in this embodiment includes a nucleotide base sequence shown in SEQ ID NO:46 from base 1 to base 9416.

As used herein, the term "vector" refers to a DNA molecule capable of autonomous replication in a cell and to which another DNA segment can be operatively linked so as to bring about replication of the attached segment. Typical vectors are plasmids,

bacteriophages and the like. Vectors capable of directing the expression of a NANBV structural protein or fusion protein are referred to herein as "expression vectors". Thus, a recombinant DNA molecule (rDNA) is a hybrid DNA molecule comprising at least two nucleotide sequences not normally found together in nature.

The choice of vector to which a DNA segment of the present invention is operatively linked depends directly, as is well known in the art, on the functional properties desired, e.g., protein expression, and the host cell to be transformed, these being limitations inherent in the art of constructing recombinant DNA molecules. However, a vector contemplated by the present invention is at least capable of directing the replication, and preferably also expression, of the recombinant or fusion protein structural gene included in DNA segments to which it is operatively linked.

In preferred embodiments, a vector contemplated by the present invention includes a procaryotic replicon (ori); i.e., a DNA sequence having the ability to direct autonomous replication and maintenance of the recombinant DNA molecule extrachromosomally in a procaryotic host cell, such as a bacterial host cell, transformed therewith. Such replicons are well known in the art. In addition, those embodiments that include a procaryotic replicon also typically include a gene whose expression confers drug resistance to a bacterial host transformed therewith. Typical bacterial drug resistance genes for use in these vectors are those that confer resistance to ampicillin or tetracycline. Typical of such vector plasmids are pUC8, pUC9, pBR322 and pBR329 available from Biorad Laboratories, (Richmond, CA).

Those vectors that include a procaryotic replicon can also include a procaryotic promoter capable of directing the expression (transcription and translation) of the gene encoding a NANBV structural protein or fusion protein in a bacterial host cell, such as E. coli, transformed therewith. A promoter is an expression control element formed by a DNA sequence that permits binding of RNA polymerase and subsequent transcription initiation to occur. Promoter sequences compatible with bacterial hosts are typically provided in plasmid vectors containing convenient restriction sites for insertion of a DNA segment of the present invention. A typical vector is pPL-lambda available from Pharmacia, (Piscataway, NJ).

Vector plasmids having a bacterial promoter that is inducible with IPTG are the pTTQ plasmids available from Amersham (Arlington Heights, IL), and the pKK223-3 plasmid available from Pharmacia. Additional expression vectors for producing in procaryotes a cloned gene product in the form of a fusion protein are well known and commercially available.

Although the expression vectors pGEX-3X and pGEX-2T have been used as exemplary in producing the fusion proteins described herein, other functionally equivalent expression vectors can be used. Functionally equivalent vectors contain an expression promoter that is inducible by IPTG for fusion protein expression in E. coli, and a configuration such that upon insertion of the DNA segment into the vector a fusion protein is produced. Commercially available vectors functionally equivalent to the vectors pGEX-3X and pGEX-2T used herein include the pGEMEX-1 plasmid vector from Promega (Madison, WI) that produces a fusion between the amino terminal portion of the T7 gene 10 protein and the cloned insert gene, the pMAL

plasmid vectors from New England Biolabs (Beverly, MA) that produce a fusion with the maltose binding protein (MBP) encoded by the mal E gene, and the pGEX-3X and pGEX-2T plasmids from Pharmacia that produce a fusion with the enzyme glutathione-s-transferase (GST) and the cloned insert gene, respectively.

The construction and use of the pGEX-3X and pGEX-2T vectors have been described by Smith et al., Gene, 67:31-40 (1988), which reference is hereby incorporated by reference.

In particularly preferred embodiments, a fusion protein contains a GST derived polypeptide-portion as an added functional domain operatively linked to a NANBV structural antigen of this invention. Any inducible promoter driven vector, such as the vectors pTTQ, pKK223-3, pGEX-3X or pGEX-2T described above and the like, can be used to express a GST-NANBV structural protein, referred to herein as a GST:NANBV fusion protein. Thus, although the pGEX-3X and pGEX-2T vectors are described as exemplary, the DNA molecules of this invention are not to be construed as limited to these vectors, because the invention in one embodiment is directed to an rDNA for expression of a protein having NANBV structural antigens fused to GST and not drawn to the vector per se.

A variety of methods have been developed to operatively link DNA segments to vectors via complementary cohesive termini. For instance, complementary homopolymer tracts can be added to the DNA segment to be inserted and to the vector DNA. The vector and DNA segment are then joined by hydrogen bonding between the complementary homopolymeric tails to form recombinant DNA molecules.

Synthetic linkers containing one or more restriction sites provide an alternative method of

joining the DNA segment to vectors. A DNA segment generated by endonuclease restriction digestion is treated with bacteriophage T4 DNA polymerase or E. coli DNA polymerase I, enzymes that remove protruding, 3', single-stranded termini with their 3'-5' exonucleolytic activities and fill in recessed 3' ends with their polymerizing activities.

The combination of these activities therefore generates blunt-ended DNA segments. The blunt-ended segments are then incubated with a large molar excess of linker molecules in the presence of an enzyme that is able to catalyze the ligation of blunt-ended DNA molecules, such as bacteriophage T4 DNA ligase. Thus, the products of the reaction are DNA segments carrying polymeric linker sequences at their ends. These DNA segments are then cleaved with the appropriate restriction enzyme and ligated to an expression vector that has been cleaved with an enzyme that produces termini compatible with those of the DNA segment.

Synthetic linkers containing a variety of restriction endonuclease sites are commercially available from a number of sources including International Biotechnologies, Inc., New Haven, CN.

Also contemplated by the present invention are RNA equivalents of the above described recombinant DNA molecules.

D. Transformed Cells and Cultures

The present invention also relates to a host cell transformed with a recombinant DNA molecule of the present invention. The term "host cell" includes both eukaryotic and prokaryotic hosts. Preferred rDNA molecules for use in a transformed cell are those described herein above and preferably are rDNAs capable of expressing a recombinant or fusion protein. Specific preferred embodiments of transformed cells

are those which contain an rDNA molecule having one of the preferred DNA segments described herein above, and particularly cells transformed with the rDNA plasmid pGEX-3X-690:694, pGEX-3X-693:691, pGEX-3X-690:691, pGEX-3X-15:17, pGEX-3X-15:18, pGEX-2T-15:17, pGEX-2T-CAP-A, pGEX-2T-CAP-B, or pGEX-2T-CAP-A-B.

Bacterial cells are preferred procaryotic host cells and typically are a strain of E. coli, such as, for example, the E. coli strain DH5 available from Bethesda Research Laboratories, Inc., Bethesda, MD. Transformation of appropriate cell hosts with a recombinant DNA molecule of the present invention is accomplished by well known methods that typically depend on the type of vector used. With regard to transformation of procaryotic host cells, see, for example, Cohen et al., Proc. Natl. Acad. Sci. USA, 69:2110 (1972); and Sambrook et al., Molecular Cloning, A Laboratory Manual, 2nd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY (1989).

Successfully transformed cells, i.e., cells that contain a recombinant DNA molecule of the present invention, can be identified by well known techniques. For example, cells resulting from the introduction of an rDNA of the present invention can be isolated as single colonies. Cells from those colonies can be harvested, lysed and their DNA content examined for the presence of the rDNA using a method such as that described by Southern, J. Mol. Biol., 98:503 (1975) or Berent et al., Biotech., 3:208 (1985).

In addition to directly assaying for the presence of rDNA, cells transformed with the appropriate rDNA can be identified by well known immunological methods when the rDNA is capable of directing the expression of a NANBV structural protein. For example, cells successfully transformed with an expression vector of

this invention produce proteins displaying NANBV structural protein antigenicity. Samples of cells suspected of being transformed are harvested and assayed for the presence of a NANBV structural antigen using antibodies specific for that antigen, such antibodies being described further herein.

Thus, in addition to the transformed host cells themselves, the present invention also contemplates a culture of those cells, preferably a monoclonal (clonally homogeneous) culture, or a culture derived from a monoclonal culture, in a nutrient medium. Preferably, the culture also contains a protein displaying NANBV structural protein antigenicity.

Nutrient media useful for culturing transformed host cells are well known in the art and can be obtained from several commercial sources.

E. Methods for Producing NANBV Structural Proteins, Polypeptides and Fusion Proteins

Another aspect of the present invention pertains to a method for producing recombinant proteins and fusion proteins of this invention.

The present method entails initiating a culture comprising a nutrient medium containing host cells, preferably E. coli cells, transformed with a recombinant DNA molecule of the present invention that is capable of expressing a NANBV structural protein or a fusion protein. The culture is maintained for a time period sufficient for the transformed cells to express the NANBV structural protein or fusion protein. The expressed protein is then recovered from the culture.

Expression vectors and expression vector culturing conditions for producing NANBV structural proteins are generally well known in the art. Such vectors and culturing conditions can be altered

without affecting the spirit of the present invention. However, preferred are the vectors designed specifically for the production of proteins not normally found in the host cell used to express a NANBV structural protein. Exemplary are the vectors that contain inducible promoters for directing the expression of DNA segments that encode the NANBV structural protein. Vectors with promoters inducible by IPTG are also well known. See for example plasmids pTTQ and pKK223-3 available from Amersham and Pharmacia respectively. Particularly preferred are the promoters inducible by IPTG present in the pGEX vectors pGEX-3X and pGEX-2T described herein.

Using vectors with inducible promoters, expression of NANBV structural proteins requires an induction phase at the beginning of the above described maintenance step for expressing the protein, as is known and described in detail in Example 2.

Methods for recovering an expressed protein from a culture are well known in the art and include fractionation of the protein-containing portion of the culture using well known biochemical techniques. For instance, the methods of gel filtration, gel chromatography, ultrafiltration, electrophoresis, ion exchange, affinity chromatography and the like, such as are known for protein fractionations, can be used to isolate the expressed proteins found in the culture. In addition, immunochemical methods, such as immunoaffinity, immunoadsorption and the like can be performed using well known methods.

Particularly preferred are isolation methods that utilize the presence of the polypeptide portion defining glutathione-S-transferase (GST) as a means to separate the fusion protein from complex mixtures of protein. Affinity adsorption of a GST-containing

fusion protein to a solid phase containing glutathione affixed thereto can be accomplished as described by Smith et al., Gene, 67:31 (1988). Alternatively, the GST-containing polypeptide portion of the fusion protein can be separated from the NANBV structural antigen by selective cleavage of the fusion protein at a specific proteolytic cleavage site, according to the methods of Smith et al., Gene, 67:31 (1988). Exemplary isolation methods are described in Examples 5 and 6.

In addition to its preparation by the use of a rDNA expression vector, a NANBV structural protein comprising a NANBV structural antigen can be prepared in the form of a synthetic polypeptide.

Polypeptides can be synthesized by any of the techniques that are known to those skilled in the polypeptide art. Synthetic chemistry techniques, such as a solid-phase Merrifield-type synthesis, are preferred for reasons of purity, antigenic specificity, freedom from undesired side products, ease of production and the like, and can be carried out according to the methods described in Merrifield et al., J. Am. Chem. Soc., 85:2149-2154 (1963) and Houghten et al., Int. J. Pept. Prot. Res., 16:311-320 (1980). An excellent summary of the many techniques available can be found in J.M. Steward and J.D. Young, "Solid Phase Peptide Synthesis", W.H. Freeman Co., San Francisco, 1969; M. Bodanszky, et al., "Peptide Synthesis", John Wiley & Sons, Second Edition, 1976 and J. Meienhofer, "Hormonal Proteins and Peptides", Vol. 2, p. 46, Academic Press (NY), 1983 for solid phase peptide synthesis, and E. Schroder and K. Kubke, "The peptides", Vol. 1, Academic Press (New York), 1965 for classical solution synthesis, each of which is incorporated herein by reference.

Appropriate protective groups usable in such synthesis are described in the above texts and in J.F.W. McOmie, "Protective Groups in Organic Chemistry", Plenum Press, New York, 1973, which is incorporated herein by reference.

A subject polypeptide includes any chemical derivative of a polypeptide whose amino acid residue sequence is shown herein. Therefore, a present polypeptide can be subject to various changes where such changes provide for certain advantages in its use.

"Chemical derivative" refers to a subject polypeptide having one or more residues chemically derivatized by reaction of a functional side group. Such derivatized molecules include for example, those molecules in which free amino groups have been derivatized to form amine hydrochlorides, p-toluene sulfonyl groups, carbobenzoxy groups, t-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatized to form salts, methyl and ethyl esters or other types of esters or hydrazides. Free hydroxyl groups may be derivatized to form O-acyl or O-alkyl derivatives. The imidazole nitrogen of histidine may be derivatized to form N-im-benzylhistidine. Also included as chemical derivatives are those peptides which contain one or more naturally occurring amino acid derivatives of the twenty standard amino acids. For examples: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine; and ornithine may be substituted for lysine. Polypeptides of the present invention also include any polypeptide having one or more additions relative to

the sequence of a polypeptide whose sequence is shown herein, so long as the requisite activity is maintained.

Additional residues may also be added at either terminus for the purpose of providing a "linker" by which the polypeptides of this invention can be conveniently affixed to a label or solid matrix, or carrier. Preferably the linker residues do not form NANBV structural antigens.

Labels, solid matrices and carriers that can be used with the polypeptides of this invention are described herein below.

Amino acid residue linkers are usually at least one residue and can be 40 or more residues, more often 1 to 10 residues, but do not form NANBV epitopes. Typical amino acid residues used for linking are tyrosine, cysteine, lysine, glutamic and aspartic acid, or the like. In addition, a subject polypeptide can differ, unless otherwise specified, from the natural sequence of the NANBV polyprotein by the sequence being modified by terminal-NH_2 acylation, e.g., acetylation, or thioglycolic acid amidation, by terminal-carboxlyamidation, e.g., with ammonia, methylamine, and the like.

When coupled to a carrier to form what is known in the art as a carrier-hapten conjugate, a polypeptide of the present invention is capable of inducing antibodies that immunoreact with NANBV. In view of the well established principle of immunologic cross-reactivity, the present invention therefore contemplates antigenically related variants of the polypeptides described herein. An "antigenically related variant" is a subject polypeptide that is capable of inducing antibody molecules that

immunoreact with a polypeptide of this invention and with NANBV.

Any peptide of the present invention may be used in the form of a pharmaceutically acceptable salt. Suitable acids which are capable of forming salts with the peptides of the present invention include inorganic acids such as hydrochloric acid, hydrobromic acid, perchloric acid, nitric acid, thiocyanic acid, sulfuric acid, phosphoric acetic acid, propionic acid, glycolic acid, lactic acid, pyruvic acid, oxalic acid, malonic acid, succinic acid, maleic acid, fumaric acid, anthranilic acid, cinnamic acid, naphthalene sulfonic acid, sulfanilic acid or the like.

Suitable bases capable of forming salts with the peptides of the present invention include inorganic bases such as sodium hydroxide, ammonium hydroxide, potassium hydroxide and the like; and organic bases such as mono-, di- and tri-alkyl and aryl amines (e.g. triethylamine, diisopropyl amine, methyl amine, dimethyl amine and the like) and optionally substituted ethanolamines (e.g. ethanolamine, diethanolamine and the like).

In general, the solid-phase synthesis methods contemplated comprise the sequential addition of one or more amino acid residues or suitably protected amino acid residues to a growing peptide chain. Normally, either the amino or carboxyl group of the first amino acid residue is protected by a suitable, selectively removable protecting group. A different, selectively removable protecting group is utilized for amino acids containing a reactive side group such as lysine.

Using a solid phase synthesis as exemplary, the protected or derivatized amino acid is attached to an inert solid support through its unprotected carboxyl

or amino group. The protecting group of the amino or carboxyl group is then selectively removed and the next amino acid in the sequence having the complimentary (amino or carboxyl) group suitably protected is admixed and reacted under conditions suitable for forming the amide linkage with the residue already attached to the solid support. The protecting group of the amino or carboxyl group is then removed from this newly added amino acid residue, and the next amino acid (suitably protected) is then added, and so forth. After all the desired amino acids have been linked in the proper sequence, any remaining terminal and side group protecting groups (and solid support) are removed sequentially or concurrently, to afford the final polypeptide.

F. NANBV Structural Protein and Fusion Protein Compositions

In another embodiment, the present invention contemplates a composition containing a NANBV structural protein, preferably isolated, comprising an amino acid residue sequence that defines a NANBV structural antigen of this invention.

By isolated is meant that a NANBV structural protein of this invention is present in a composition as a major protein constituent, typically in amounts greater than 10% of the total protein in the composition, but preferably in amounts greater than 90% of the total protein in the composition.

A NANBV structural antigen, as used herein, is a structural protein coded by the genome of NANBV and has the properties of an antigen as defined herein, namely, to be able to immunoreact specifically with an antibody. NANBV structural proteins have been tentatively designated as capsid and envelope, and have been partially characterized as described herein

to contain the NANBV structural antigens capsid and envelope, respectively.

NANBV capsid antigen as described herein comprises an amino acid residue sequence that is immunologically related in sequence to the putative Hutch strain NANBV capsid antigen, whose sequence is contained in SEQ ID NO:1 from residue 1 to residue 120.

NANBV envelope antigen as described herein comprises an amino acid residue sequence that is immunologically related in sequence to the putative Hutch strain NANBV envelope antigen, a portion of whose sequence is contained in SEQ ID NO:1 from residue in 121 to residue 326.

By "immunologically related" is meant that sufficient homology in amino acid sequence is present in the two protein sequences being compared that antibodies specific for one protein immunoreact (cross-react) with the other protein. Immunological cross-reactivity can be measured by methods well known including the immunoassay methods described herein.

As used herein, the phrase "recombinant protein" refers to a protein of at least 20 amino acid residues in length, and preferably at least 50 residues, that includes an amino acid residue sequence that corresponds, and preferably is identical, to a portion of the NANBV structural protein contained in SEQ ID NO:1.

In preferred embodiments a NANBV structural protein includes an amino acid residue sequence that is immunologically related to, and preferably is identical to, the sequence contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, or from residue 1 to residue 74. The NANBV structural protein

with the indicated sequence is particularly preferred for use in diagnostic methods and systems because the capsid antigens contained therein were demonstrated herein to be particularly useful in detecting acute NANBV infection. Related NANBV structural proteins include a sequence contained in SEQ ID NO:1 from residue 1 to residue 120, from residue 1 to residue 176, and from residue 1 to residue 326. Exemplary are the proteins described herein having a sequence contained in SEQ ID NO:2 from residue 1 to residue 315, in SEQ ID NO:3 from residue 1 to residue 252, in SEQ ID NO:4 from residue 1 to residue 252, or in SEQ ID NO:6 from residue 1 to residue 271.

In another embodiment a NANBV structural protein includes an amino acid residue sequence that is immunologically related to, and preferably is identical to, the sequence contained in SEQ ID NO:1 from residue 69 to residue 120. An exemplary NANBV structural protein has the sequence of the expressed protein coded for by the rDNA plasmid pGEX-3X-693:691.

Additional NANBV structural proteins containing NANBV envelope antigen are contemplated that include an amino acid residue sequence that is immunologically related to, and preferably is identical to, the sequence contained in SEQ ID NO:1 from residue 121 to residue 176. Exemplary are the proteins having a sequence of the expressed protein coded for by one of the rDNA plasmids pGEX-3X-15:17, pGEX-3X-15:18 and pGEX-2T-15:17.

In another embodiment, a NANBV structural protein is contemplated that comprises an amino acid residue sequence according to a polypeptide of this invention.

In preferred embodiments a NANBV structural protein is essentially free of both procaryotic antigens (i.e., host cell-specific antigens) and other

NANBV-related proteins. By "essentially free" is meant that the ratio of NANBV structural antigen to foreign antigen, such as procaryotic antigen, or other NANBV-related protein is at least 10:1, preferably is 100:1, and more preferably is 200:1.

The presence and amount of contaminating protein in a NANBV structural protein preparation can be determined by well known methods. Preferably, a sample of the composition is subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) to separate the NANBV structural protein from any protein contaminants present. The ratio of the amounts of the proteins present in the sample is then determined by densitometric soft laser scanning, as is well known in the art. See Guilian et al., Anal. Biochem., 129:277-287 (1983).

A NANBV structural protein can be prepared as an isolated protein, and more preferably essentially free of procaryotic antigens or NANBV non-structural antigens by the methods disclosed herein for producing NANBV structural proteins. Particularly preferred are methods which rely on the properties of a polypeptide region of a fusion protein, which region is present in the fusion protein to facilitate separation of the fusion protein from host cell proteins on the basis of affinity. Exemplary are the GST-containing fusion proteins whose amino acid residue sequences are contained in SEQ ID NOS:2, 3, 4 or 6 wherein the GST polypeptide region of each provides the fusion protein with a functional domain having an affinity to bind to the normal substrate for GST, namely glutathione. The purification of a fusion protein having a GST polypeptide region is described further herein.

In a related embodiment, the invention describes a polypeptide that defines a NANBV antigen. Thus, the

invention contemplates a polypeptide corresponding to a region of the NANBV polyprotein that defines an antigenic determinant of the virus that is useful as a NANBV antigen in serological assays or in an inoculum to induce anti-NANBV antisera, as described herein.

A polypeptide of this invention comprises a sequence of amino acids of about 7 to about 200 residues in length, preferably about 20 to 150 residues in length, that comprises an amino acid residue sequence defined by the nucleotide sequence of a polynucleotide of this invention.

A preferred polypeptide comprises an amino acid residue sequence that includes an amino acid residue sequence selected from the group of sequences consisting of residue 391 to residue 404 of SEQ ID NO:46, residue 246 to residue 256 of SEQ ID NO:46, residue 461 to residue 466 of SEQ ID NO:46, residue 473 to residue 482 of SEQ ID NO:46, and residue 2356 to residue 2379 of SEQ ID NO:46. In particularly preferred embodiments the polypeptide has an amino acid residue sequence that corresponds to the sequence shown in SEQ ID NO:46.

Insofar as a polypeptide is useful to distinguish Hutch isolates, the invention contemplates a polypeptide having a length from about 7 to about 200 amino acid residues and comprising an amino acid residue sequence that corresponds to a portion of the sequence of the Hutch c59 isolate of NANBV shown in SEQ ID NO:46. In this embodiment, the polypeptide has at least one amino acid residue difference in sequence when compared to the amino acid residue sequence of an isolate of NANBV selected from the group consisting of HCV-1, HCV-BK, HCV-J, HC-J1, HC-J4, HCV-JH and HCV-Hh.

Preferably, a polypeptide is immunoreactive with anti-Hutch strain NANBV antisera when measured in

standard serological immunoassays such as are described herein.

More preferably, a polypeptide contains at least one amino acid residue sequence difference in a variable region of the NANBV viral genome-encoded polyprotein as defined herein, such as an amino acid residue sequence that is selected from the group of sequences consisting of the V variable region amino acid residue sequence (residue 386 to residue 411 of SEQ ID NO:46), the V_1 variable region amino acid residue sequence (residue 246 to residue 275 of SEQ ID NO:46), the V_2 variable region amino acid residue sequence (residue 456 to residue 482 of SEQ ID NO:46), and the V_3 variable region amino acid residue sequence (residue 2356 to residue 2379 of SEQ ID NO:46).

In another embodiment, a composition comprising an isolated fusion protein is also contemplated by the present invention that comprises a NANBV structural protein of this invention operatively linked at one or both termini to another polypeptide by a peptide bond. The added polypeptide can be any polypeptide designed to increase the functional domains present on the fusion protein. The added functional domains are included to provide additional immunogenic epitopes, to add mass to the fusion protein, to alter the solubility of the fusion protein, to provide a means for affinity-based isolation of the fusion protein, and the like. Exemplary added functional domains are the Thrombin or Factor Xa specific cleavage sites provided when a subject fusion protein is produced in the vector pGEX-3X or pGEX-2T, respectively, as described herein. An additional exemplary domain is the GST-derived protein domain that allows rapid isolation using affinity chromatography to a solid phase containing glutathione affixed thereto.

A Thrombin or Factor Xa cleavage site-containing domain is used herein, in one embodiment, to allow production of an NANBV structural protein free of the GST function domain. Exemplary is the protein produced in Example 6 having an amino acid residue sequence contained in SEQ ID NO:2 from residue 226 to residue 315. The Factor Xa cleavage site-containing domain is also used in the commercially available fusion protein expression vector pMAL available from New England Biolabs (Beverly, MA) described herein.

In a related embodiment a NANBV structural protein is produced by Thrombin cleavage of a protein produced using the pGEX-2T vector, such as a protein having an amino acid residue sequence contained in SEQ ID NO:3 from residue 225 to residue 252, in SEQ ID NO:4 from residue 225 to residue 252, or in SEQ ID NO:6 from residue 225 to residue 271.

A fusion protein of the present invention includes an amino acid residue sequence corresponding from its amino-terminus to its carboxy-terminus to the amino acid residue sequence contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, from residue 1 to residue 74, from residue 69 to residue 120, from residue 121 to residue 176, or from residue 121 to residue 326. A preferred fusion protein has a sequence corresponding to, and more preferably is identical to, the amino acid residue sequence in SEQ ID NO:2 from residue 1 to residue 315, in SEQ ID NO:3 from residue 1 to residue 252, in SEQ ID NO:4 from residue 1 to residue 252, or in SEQ ID NO:6 from residue 1 to residue 271. Other preferred fusion proteins are defined by the amino acid residue sequence of the expressed protein coding sequence present in the rDNA plasmids pGEX-3X-690:694, pGEX-3X-

690:691, pGEX-3X-693:691, pGEX-3X-15:17, pGEX-3X-15:18, pGEX-2T-15:17, pGEX-2T-CAP-A, pGEX-2T-CAP-B, and pGEX-2T-CAP-A-B.

The phrase "fusion protein", when used herein refers to an isolated protein as it was defined for a NANBV structural protein of this invention. Thus an isolated fusion protein is a composition having a fusion protein of this invention in amounts greater than 10 percent of the total protein in the composition, and preferably greater than 90 percent of the total protein in the composition.

A preferred fusion protein is a heterologous fusion protein, that is, a fusion protein that contains a polypeptide portion derived from a protein originating in a heterologous species of virus, organism, pathogen or animal, i.e., a non-NANBV protein. Preferably a heterologous fusion protein contains a non-NANBV polypeptide portion that is not immunologically related to a NANBV structural antigen of this invention.

In one embodiment, a fusion protein contains a functional domain that provides an immunogenic or antigenic epitope other than the NANBV structural antigen defined herein and is preferably derived from a separate pathogen, or from several pathogens. The functional domain is immunogenic where that domain is present to form a polyvalent vaccine or immunogen for the purpose of inducing antibodies immunoreactive with both NANBV structural protein and a second pathogen. The functional domain is antigenic where that domain is present to form a polyvalent antigen for use in diagnostic systems and methods for detecting at least two species of antibodies.

Of particular interest in this embodiment are fusion proteins designed to include a functional

domain that is derived from other hepatitis-causing viruses, such as Hepatitis B virus, and Hepatitis A virus. These viruses have been well characterized to contain antigenic determinants and immunogenic determinants suitable for use in the fusion protein of this invention, and provide the advantage of multipurpose biochemical reagents in both diagnostic and vaccine applications. Additionally, the included functional domain can contain amino acid sequences from other pathogens, preferably those which may also infect individuals with NANBV hepatitis, such as HIV.

Preferred NANBV structural proteins or fusion proteins comprising a NANBV structural antigen of the present invention are in non-reduced form, i.e., are substantially free of sulfhydryl groups because of intramolecular Cys-Cys bonding.

In preferred compositions, the NANBV structural protein or fusion protein as described herein, is present, for example, in liquid compositions such as sterile suspensions or solutions, or as isotonic preparations containing suitable preservatives.

One such composition useful for inducing anti-NANBV structural protein antibodies in a mammal is referred to as a vaccine and contains a NANBV structural protein or fusion protein of this invention.

G. Vaccines

1. Introduction

The word "vaccine" in its various grammatical forms is used herein to describe a type of inoculum containing one or more NANBV structural antigens of this invention as an active ingredient in a pharmaceutically acceptable excipient that is used to induce production of antibodies in a mammal

immunoreactive with NANBV, and preferably induce active immunity in a host mammal against NANBV.

An inoculum comprises, as an active immunogenic ingredient, an immunologically effective amount of at least one NANBV structural protein, polypeptide cr fusion protein of this invention, or a combination thereof.

Because an inoculum is typically designed to induce specific antibodies, it is preferred that an inoculum contains a NANBV structural protein comprised of only NANBV structural antigens and not other functional domains as described for a fusion protein. Thus a preferred inoculum contains a NANBV structural protein of this invention that includes an amino acid residue sequence contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, from residue 1 to residue 74, from residue 69 to residue 120, from residue 121 to residue 176, or from residue 121 to residue 326. Particularly preferred as an active ingredient in an inoculum is a NANBV structural protein having the amino acid residue sequence contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, from residue 1 to residue 74, from residue 1 to residue 120, or contained in SEQ ID NO:2 from residue 226 to residue 315, contained in SEQ ID NO:3 from residue 225 to residue 252, contained in SEQ ID NO:4 from residue 225 to residue 252, or contained in SEQ ID NO:6 from residue 225 to residue 271.

A preferred inoculum comprises the entire E_1 domain and E_2/NS1 domain encoded by a DNA sequence spanning nucleotides 571 to 2197 in SEQ ID NO:46.

An inoculum can contain one or more polypeptides of this invention as an active ingredient. Such

inoculums are particularly useful to produce an antibody immunoreactive with NANBV because the polypeptide can be designed to define a small and therefore unique epitope of the NANBV polyprotein. Such antibodies are isolate-specific as defined herein.

Alternatively, a polyvalent inoculum is contemplated that comprises a fusion protein that has more than 1 immunogenic functional domains and is useful to induce classes of antibodies specific for different antigens; namely a first NANBV structural antigen as described herein, or correspondence regions from different strains of HCV and a further antigen present on a distinct pathogen. Preferred further antigens are derived from pathogens that are typically found in association with NANBV-infected patients, namely Hepatitis B Virus, Human Immunodeficiency Virus (HIV) and the like.

A related embodiment contemplates two immunogenic domains, each from a different region of HCV, such that a single inoculum induces antibodies specific for two regions of the HCV encoded polyprotein.

2. Preparation

The preparation of an inoculum that contains a protein or polypeptide as an active ingredient is well understood in the art. Typically, such inoculums are prepared as injectables, either as liquid solutions or suspensions; solid forms suitable for solution in, or suspension in, liquid prior to injection may also be prepared. The preparation can also be emulsified.

The active immunogenic ingredient is dissolved, dispersed or admixed in an excipient that is pharmaceutically acceptable and compatible with the active ingredient as is well known. The phrases "suitable for human use" and "pharmaceutically

acceptable" (physiologically tolerable) refer to molecular entities and compositions that typically do not produce an allergic or similar untoward reaction, such as gastric upset, dizziness and the like, when administered to a human. Suitable excipients may take a wide variety of forms depending on the intended use and are, for example, aqueous solutions containing saline, phosphate buffered saline (PBS), dextrose, glycerol, ethanol, or the like and combinations thereof. In addition, if desired, the inoculum can contain minor amounts of auxiliary substances such as wetting or emulsifying agents, pH buffering agents, mineral oils, carriers or adjuvants which enhance the effectiveness of the inoculum. A preferred embodiment contains at least about 0.01% to about 99% of NANBV structural protein or fusion as an active ingredient, typically at a concentration of about 10 to 200 μg of active ingredient per ml of excipient.

3. Carriers

An inoculum may comprise a polypeptide or NANBV structural protein of this invention linked to a carrier, or an antigenic carrier, to facilitate the production of an immune response in the immunized mammal.

One or more additional amino acid residues may be added to the amino- or carboxy-termini of the NANBV structural protein to assist in binding the protein to a carrier if not already present on the protein. Cysteine residues added at the amino- or carboxy-termini of the protein have been found to be particularly useful for forming polymers via disulfide bonds. However, other methods well known in the art for preparing conjugates can also be used. Exemplary additional linking procedures include the use of Michael addition reaction products, dialdehydes such

as glutaraldehyde, Klipstein et al., J. Infect. Dis., 147:318-326 (1983) and the like, or the use of carbodiimide technology as in the use of a water-soluble carbodiimide to form amide links to the carrier.

Useful carriers are well known in the art, and are generally proteins themselves. Exemplary of such carriers are keyhole limpet hemocyanin (KLH), edestin, thyroglobulin, albumins such as bovine serum albumin (BSA) or human serum albumin (HSA), red blood cells such as sheep erythrocytes (SRBC), tetanus toxoid, cholera toxoid as well as poly amino acids such as poly (D-lysine: D-glutamic acid), and the like.

As is also well known in the art, it is often beneficial to bind a NANBV structural protein to its carrier by means of an intermediate, linking group. As noted above, glutaraldehyde is one such linking group. However, when cysteine is used, the intermediate linking group is preferably an m-maleimidobenzoic acid N-hydroxysuccinimide ester (MBS).

Additionally, MBS may be first added to the carrier by an ester-amide interchange reaction. Thereafter, the addition can be followed by addition of a blocked mercapto group such as thiolacetic acid (CH_3COSH) across the maleimido-double bond. After cleavage of the acyl blocking group, a disulfide bond is formed between the deblocked linking group mercaptan and the mercaptan of the cysteine residue of the protein.

Antigenic carriers can be utilized to potentiate or boost the immune response (immunopotentiation), or to direct the type of immune response by use of the inoculum in combination with the carrier. See, for example, the teachings of Milich et al., in U.S.

Patent Nos. 4,599,231, 4,599,230 and 4,683,136, and the teachings of Thornton et al., in U.S. Patent Nos. 4,818,527 and 4,882,145.

Other means of immunopotentiation include the use of liposomes and immuno-stimulating complex (ISCOM) particles. The unique versatility of liposomes lies in their size adjustability, surface characteristics, lipid composition and ways in which they can accommodate antigens. Methods to form liposomes are known in the art. See, for example, Prescott, Ed., Methods in Cell Biology, Vol. XIV, Academic Press, NY (1976) p.33 et seq. In ISCOM particles, the cage-like matrix is composed of Quil A, extracted from the bark of a South American tree. A strong immune response is evoked by antigenic proteins or peptides attached by hydrophobic interaction with the matrix surface.

The choice of carrier is more dependent upon the ultimate use of the immunogen than upon the determinant portion of the immunogen, and is based upon criteria not particularly involved in the present invention. For example, if an inoculum is to be used in animals, a carrier that does not generate an untoward reaction in the particular animal should be selected.

4. Administration

An inoculum is conventionally administered parenterally, by injection, for example, either subcutaneously or intramuscularly. Additional formulations which are suitable for other modes of administration include suppositories and, in some cases, oral formulations. For suppositories, traditional binders and carriers may include, for example, polyalkylene glycols or triglycerides; such suppositories may be formed from mixtures containing the active ingredient in the range of 0.5% to 10%,

preferably 1-2%. Oral formulations include such normally employed excipients as, for example, pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate and the like. The compositions take the form of solutions, suspensions, tablets, pills, capsules, sustained release formulations or powders and contain 10%-95% of active ingredient, preferably 25-70%.

A NANBV structural protein can be formulated into an inoculum as a neutral or salt form. Pharmaceutically acceptable salts, include the acid addition salts (formed with the free amino groups of the antigen) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine, and the like.

The inoculum is administered in a manner compatible with the dosage formulation, and in such amount as will be immunogenic and effective to induce an immune response. The quantity of inoculum to be administered to achieve desired full protective immunity when used as a vaccine depends on the subject to be immunized, capacity of the subject's immune system to synthesize antibodies or induce cell-mediated response, and the degree of protection desired. Precise amounts of active ingredient required to be administered depend on the judgement of the practitioner and are peculiar to each individual, but generally a dosage suitable for a broad population

can be defined. Suitable dosage ranges are of the order of about ten micrograms (μg) to several milligrams (mg), preferably about 10-500 micrograms and more preferably about 100 micrograms active ingredient for each single immunization dose for a human adult. Suitable regimes for initial administration and booster shots are also variable, but are typified by an initial administration followed in two to six week intervals by a subsequent injection or other administration.

An inoculum can also include an adjuvant as part of the excipient. Adjuvants such as complete Freund's adjuvant (CFA), incomplete Freund's adjuvant (IFA) for use in laboratory mammals are well known in the art. Pharmaceutically acceptable adjuvants such as alum can also be used. An exemplary inoculum thus comprises one ml of phosphate buffered saline (PBS) containing about 50 to 200 μg NANBV structural protein or polypeptide adsorbed onto about 0.5 mg to about 2.5 mg of alum, or to 0.1% to 1% $Al(OH)_3$. A preferred inoculum comprises 1 ml of PBS containing 100 μg NANBV structural protein adsorbed onto 2.5 mg of alum carrier.

After administration of the inoculum, the mammal or human receiving the inoculum is maintained for a time period sufficient for the immune system of the mammal to respond immunologically, typically on the order of 2 to 8 weeks, as is well known, by the production of antibodies immunoreactive with the immunogen.

H. Antibody Compositions

An antibody of the present invention is a composition containing antibody molecules that immunoreact with a NANBV structural antigen, with the Hutch isolate of NANBV, preferably the c59 isolate,

and with a NANBV structural protein, polypeptide or fusion protein of the present invention (anti-NANBV structural protein antibody molecules). A preferred antibody contains antibody molecules that immunoreact with an epitope present on a polypeptide having an amino acid residue sequence contained in SEQ ID NO:1 from residue 1 to residue 326, preferably that immunoreacts with a polypeptide having the sequence contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, from residue 1 to residue 74, from residue 49 to residue 120, or from residue 121 to residue 326.

In addition, it is preferred that anti-NANBV structural protein antibody molecules do not immunoreact with the NANBV isolates HCV-1, HCV-BK, HCV-J, HC-J1, HC-J4, HCV-JH or HCV-Hh, or with the C-100-3 antigen described herein, and available in the commercial assay available from Ortho Diagnostics, Inc.

An antibody of the present invention is typically produced by immunizing a mammal with an inoculum containing Hutch c59 isolate or a NANBV structural protein or polypeptide of this invention and thereby induce in the mammal antibody molecules having immunospecificity for the NANBV structural antigens described herein. The antibody molecules are then collected from the mammal and isolated to the extent desired by well known techniques such as, for example, by using DEAE Sephadex to obtain the IgG fraction.

To enhance the specificity of the antibody, the antibodies may be purified by immunoaffinity chromatography using solid phase-affixed immunizing NANBV structural protein. The antibody is contacted with the solid phase-affixed NANBV structural protein for a period of time sufficient for the NANBV

structural protein to immunoreact with the antibody molecules to form a solid phase-affixed immunocomplex. The bound antibodies are separated from the complex by standard techniques.

To produce an antibody composition that does not immunoreact with the C-100-3 antigen or the NANBV isolates identified above, immunoadsorption methods are used to remove the undesirable immunospecificities. Immunoadsorption methods to remove immunospecificities are generally well known and involve first contacting the antibody composition with a solid phase having affixed thereto one or more of the antigens or NANBV isolates to form an immunoadsorption admixture. Preferably, there is an excess of antigen or NANBV in the solid phase in proportion to the antibodies in the composition having the undesirable immunospecificities in the immunoadsorption admixture.

The immunoadsorption admixture is then maintained under immunoreaction conditions and for a time period sufficient for an immunocomplex to form in the solid phase. Thereafter, the liquid and solid phases are separated, and the liquid phase is retained having the undesirable antibody molecules immunoadsorbed away onto the solid phase.

Particularly preferred is an antibody composition containing c59 isolate specific antisera, formed by immunization with Hutch c59 isolate, or preferably with a polypeptide of this invention selected as defined herein to have an amino acid residue sequence unique to c59 and preferably derived from the V, V1, V2 or V3 variable regions of NANBV. Thereafter, the produced antibody composition is immunoadsorbed to remove antibodies immunoreactive with NANBV isolates other than c59 as described herein.

The antibody so produced can be used, inter alia, in the diagnostic methods and systems of the present invention to detect NANBV structural antigens as described herein present in a body sample.

The word "inoculum" in its various grammatical forms is used herein to describe a composition containing a NANBV structural antigen of this invention as an active ingredient used for the preparation of antibodies immunoreactive with NANBV structural antigens.

The preparation and use of an inoculum for production of an antibody of this invention largely parallels the descriptions herein for a vaccine insofar as the vaccine is also designed to induce the production of antibodies and is exemplary of the preparation and use of an inoculum. A key difference is that the inoculum is formulated for use on an animal rather than a human, as is well known.

A preferred antibody is a monoclonal antibody and can be used in the same manner as disclosed herein for antibodies of the present invention.

A monoclonal antibody is typically composed of antibodies produced by clones of a single cell called a hybridoma that secretes (produces) but one kind of antibody molecule. The hybridoma cell is formed by fusing an antibody-producing cell and a myeloma or other self-perpetuating cell line. The preparation of such antibodies were first described by Kohler and Milstein, Nature 256:495-497 (1975), which description is incorporated by reference. The hybridoma supernates so prepared can be screened for immunoreactivity with a NANBV structural antigen such as the NANBV structural protein used in the inoculum to induce the antibody-producing cell. Other methods

of producing monoclonal antibodies, the hybridoma cell, and hybridoma cell cultures are also well known.

Also contemplated by this invention is the hybridoma cell, and cultures containing a hybridoma cell that produce a monoclonal antibody of this invention.

It should be understood that in addition to the aforementioned carrier ingredients the pharmaceutical formulation described herein can include, as appropriate, one or more additional carrier ingredients such as diluents, buffers, binders, surface active agents, thickness, lubricants, preservatives (including antioxidants) and the like, and substances included for the purpose of rendering the formulation isotonic with the blood of the intended recipient. Typically, a preservative such as merthiolate (at a 1:5000 dilution of a 1% solution) is added to eliminate the risk of microbial contamination, even if sterile techniques were employed in the manufacture of the inoculum.

I. Diagnostic Systems and Methods

1. Diagnostic Systems

The present invention contemplates a diagnostic system for assaying for the presence of anti-NANBV antibodies or NANBV structural antigens in a body sample according to the diagnostic methods described herein.

A diagnostic system in kit form includes, in an amount sufficient for at least one assay according to the methods described herein, a NANBV structural protein, polypeptide or fusion protein or a combination thereof of the present invention, or an anti-NANBV antibody composition of this invention, as a separately packaged reagent. Instructions for use of the packaged reagent are also typically included.

"Instructions for use" typically include a tangible expression describing the reagent concentration or at least one assay method parameter such as the relative amounts of reagent and sample to be admixed, maintenance time periods for reagent/ sample admixtures, temperature, buffer conditions and the like.

In preferred embodiments, a diagnostic system of the present invention further includes a label or indicating means capable of signaling the formation of a complex containing a NANBV structural antigen, a recombinant protein or an anti-NANBV antibody.

As used herein, the terms "label" and "indicating means" in their various grammatical forms refer to single atoms and molecules that are either directly or indirectly involved in the production of a detectable signal to indicate the presence of a complex. Any label or indicating means can be linked to or incorporated in a reagent species such as an antibody or monoclonal antibody, or can be used separately, and those atoms or molecules can be used alone or in conjunction with additional reagents. Such labels are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel proteins, methods and/or systems.

The label can be a fluorescent labeling agent that chemically binds to antibodies or antigens without denaturing them to form a fluorochrome (dye) that is a useful immunofluorescent tracer. Suitable fluorescent labeling agents are fluorochromes such as fluorescein isocyanate (FIC), fluorescein isothiocyanite (FITC), 5-dimethylamine-1-naphthalenesulfonyl chloride (DANSC), tetramethylrhodamine isothiocyanate (TRITC),

lissamine, rhodamine 8200 sulfonyl chloride (RB 200 SC), a chelate-lanthanide bound (e.g., Eu, Tb, Sm) and the like. A description of immunofluorescence analysis techniques is found in DeLuca, "Immunofluorescence Analysis", in Antibody As a Tool, Marchalonis, et al., eds., John Wiley & Sons, Ltd., pp. 189-231 (1982), which is incorporated herein by reference.

In preferred embodiments, the label is an enzyme, such as horseradish peroxidase (HRP), glucose oxidase, alkaline phosphatase or the like. In such cases where the principal label is an enzyme such as HRP or glucose oxidase, additional reagents are required to visualize the fact that an antibody-antigen complex (immunoreactant) has formed. Such additional reagents for HRP include hydrogen peroxide and an oxidation dye precursor such as diaminobenzidine. An additional reagent useful with HRP is 2,2'-azino-di-(3-ethyl-benzthiazoline-6-sulfonic acid) (ABTS).

Radioactive elements are also useful labeling agents and are used illustratively herein. An exemplary radiolabeling agent is a radioactive element that produces gamma ray emissions. Elements which themselves emit gamma rays, such as ^{124}I, ^{125}I, ^{128}I, ^{131}I and ^{51}Cr represent one class of gamma ray emission-producing radioactive element indicating groups. Particularly preferred is ^{125}I. Another group of useful labeling means are those elements such as ^{11}C, ^{18}F, ^{15}O and ^{13}N which themselves emit positrons. The positrons so emitted produce gamma rays upon encounters with electrons present in the animal's body. Also useful is a beta emitter, such as ^{111} indium, ^{3}H, ^{35}S, ^{14}C, or ^{32}P.

Additional labels have been described in the art and are suitable for use in the diagnostic systems of

this invention. For example, the specific affinity found between pairs of molecules can be used, one as a label affixed to the specific binding agent and the other as a means to detect the presence of the label. Exemplary pairs are biotin:avidin, where biotin is the label, and peroxidase:anti-peroxidase (PAP), where peroxidase is the label.

The linking of labels, i.e., labeling of, polypeptides and proteins is well known in the art. For instance, antibody molecules produced by a hybridoma can be labeled by metabolic incorporation of radioisotope-containing amino acids provided as a component in the culture medium. See, for example, Galfre et al., Meth. Enzymol., 73:3-46 (1981). The techniques of protein conjugation or coupling through activated functional groups are particularly applicable. See, for example, Aurameas, et al., Scand. J. Immunol., Vol. 8 Suppl. 7:7-23 (1978), Rodwell et al., Biotech., 3:889-894 (1984), and U.S. Pat. No. 4,493,795.

The diagnostic systems can also include, preferably as a separate package, a specific binding agent. A "specific binding agent" is a molecular entity capable of selectively binding a reagent species, which in turn is capable of reacting with a product of the present invention but is not itself a protein expression product of the present invention. Exemplary specific binding agents are antibody molecules such as anti-human IgG or anti-human IgM, complement proteins or fragments thereof, protein A, and the like. Preferably the specific binding agent can bind the anti-NANBV antibody to be detected when the antibody is present as part of an immunocomplex.

In preferred embodiments the specific binding agent is labeled. However, when the diagnostic system

includes a specific binding agent that is not labeled, the agent is typically used as an amplifying means or reagent. In these embodiments, the labeled specific binding agent is capable of specifically binding the amplifying means when the amplifying means is bound to a reagent species-containing complex.

The diagnostic kits of the present invention can be used in an "ELISA" format to detect the presence or quantity of antibodies in a body fluid sample such as serum, plasma or saliva. "ELISA" refers to an enzyme-linked immunosorbent assay that employs an antibody or antigen bound to a solid phase and an enzyme-antigen or enzyme-antibody conjugate to detect and quantify the amount of an antigen or antibody present in a sample. A description of the ELISA technique is found in Chapter 22 of the 4th Edition of Basic and Clinical Immunology by D.P. Sites et al., published by Lange Medical Publications of Los Altos, CA in 1982 and in U.S. Patents No. 3,654,090; No. 3,850,752; and No. 4,016,043, which are all incorporated herein by reference.

Thus, in preferred embodiments, the NANBV structural protein, polypeptide, fusion protein or anti-NANBV antibody of the present invention can be affixed to a solid matrix to form a solid support that is separately packaged in the subject diagnostic systems.

The reagent is typically affixed to the solid matrix by adsorption from an aqueous medium although other modes of affixation, well known to those skilled in the art, can be used.

Useful solid matrices are well known in the art. Such materials include the cross-linked dextran available under the trademark SEPHADEX from Pharmacia Fine Chemicals (Piscataway, NJ); agarose; beads of

polystyrene about 1 micron to about 5 millimeters in diameter available from Abbott Laboratories of North Chicago, IL; polyvinyl chloride, polystyrene, cross-linked polyacrylamide, nitrocellulose- or nylon-based webs such as sheets, strips or paddles; or tubes, plates or the wells of a microtiter plate such as those made from polystyrene or polyvinylchloride.

The present invention also contemplates a diagnostic system for assaying the presence of NANBV nucleic acids in a body sample using hybridization of polynucleotides or oligonucleotides of this invention to NANBV nucleic acids according to the diagnostic methods described herein.

A diagnostic system for assaying for the presence of NANBV nucleic acids in kit form includes, in an amount sufficient for at least one assay, a polynucleotide of the present invention, as a separately packaged reagent. Instructions for use of the packaged reagent are also typically included.

In preferred embodiments, a diagnostic system of this embodiment further includes a label or indicating means capable of signaling the formation of a hybridization complex containing a NANBV nucleic acid.

The NANBV structural protein, polypeptide, fusion protein, anti-NANBV antibody, polynucleotides, labeled specific binding agent or amplifying reagent of any diagnostic system described herein can be provided in solution, as a liquid dispersion or as a substantially dry power, e.g., in lyophilized form. Where the indicating means is an enzyme, the enzyme's substrate can also be provided in a separate package of a system. A solid support such as the before-described microtiter plate and one or more buffers can also be included as separately packaged elements in this diagnostic assay system.

The packages discussed herein in relation to diagnostic systems are those customarily utilized in diagnostic systems. Such packages include glass and plastic (e.g., polyethylene, polypropylene and polycarbonate) bottles, vials, plastic and plastic-foil laminated envelopes and the like.

2. Diagnostic Methods

The present invention contemplates any diagnostic method that results in detecting anti-NANBV structural protein antibodies or NANBV structural antigens in a body sample using a NANBV structural protein, polypeptide, fusion protein or anti-NANBV structural antigen antibody of this invention as an immunochemical reagent to form an immunoreaction product whose amount relates, either directly or indirectly, to the amount of material to be detected in the sample. Those skilled in the art will understand that there are numerous well known clinical diagnostic chemistry procedures in which an immunochemical reagent of this invention can be used to form an immunoreaction product whose amount relates to the amount of specific antibody or antigen present in a body sample.

Various heterogenous and homogenous protocols, either competitive or noncompetitive, can be employed in performing an assay method of this invention. Thus, while exemplary methods are described herein, the invention is not so limited.

To detect the presence of anti-NANBV structural protein antibodies in a patient, a body sample, and preferably a body fluid sample such as blood, plasma, serum, urine or saliva from the patient, is contacted by admixture under biological assay conditions with a NANBV antigenic molecule of this invention such as a NANBV structural protein, and preferably with a

polypeptide or fusion protein of the present invention, to form an immunoreaction admixture. The admixture is then maintained for a period of time sufficient to allow the formation of a NANBV antigenic molecule-antibody molecule immunoreaction product (immunocomplex). The presence, and preferably the amount, of complex can then be detected as described herein. The presence of the complex is indicative of anti-NANBV antibodies in the sample.

In preferred embodiments the presence of the immunoreaction product formed between NANBV antigenic molecules and a patient's antibodies is detected by using a specific binding reagent as discussed herein. For example, the immunoreaction product is first admixed with a labeled specific binding agent to form a labeling admixture. A labeled specific binding agent comprises a specific binding agent and a label as described herein. The labeling admixture is then maintained under conditions compatible with specific binding and for a time period sufficient for any immunoreaction product present to bind with the labeled specific binding agent and form a labeled product. The presence, and preferably amount, of labeled product formed is then detected to indicate the presence or amount of immunoreaction product.

In preferred embodiments the diagnostic methods of the present invention are practiced in a manner whereby the immunocomplex is formed and detected in a solid phase, as disclosed for the diagnostic systems herein.

Thus, in a preferred diagnostic method, the NANBV structural protein or polypeptide is affixed to a solid matrix to form the solid phase. It is further preferred that the specific binding agent is protein A, or an anti-human Ig, such as IgG or IgM, that can

complex with the anti-NANBV structural protein antibodies immunocomplexed in the solid phase with the NANBV structural protein. Most preferred is the use of labeled specific binding agents where the label is a radioactive isotope, an enzyme, biotin or a fluorescence marker such as lanthanide as described for the diagnostic systems, or detailed by references shown below.

In this solid phase embodiment, it is particularly preferred to use a recombinant protein that contains the antigen defined by the amino acid residue sequence contained in SEQ ID NO:1 from residue 1 to residue 20, from residue 21 to residue 40, from residue 2 to residue 40, or from residue 1 to residue 74, as embodied in the fusion proteins as described in Example 7.

In another preferred diagnostic method, the NANBV antigenic molecule of the invention is affixed to solid matrix as described above, and dilutions of the biological sample are subjected to the immunocomplexing step by contacting dilutions of sample with the solid surface and removing non-bound materials. Due to the multivalence of antibodies present in biological samples from infected individuals (bivalent for IgG, pentavalent for IgM) subsequent addition of labeled NANBV structural protein, polypeptide or fusion protein of the invention to this admixture will become attached to the solid phase by the sample antibody serving as bridge between the solid phase NANBV antigenic molecules of the invention and the soluble, labeled molecules. The presence of label in the solid phase indicates the presence and preferably the amount of specific antibody in the sample. One skilled in the art can determine a range of dilutions and determine

therefrom a concentration of labeled antigen in the solid phase. The biological sample and the labeled NANBV antigenic molecules of the invention can be admixed prior to, or simultaneously with contacting the biological sample with the solid phase allowing the trimolecular complex to form at the solid phase by utilizing the bridging property of bivalent or multivalent specific antibody. As a particularly useful label, biotinylated NANBV antigenic molecules of the invention can be the labeled antigen, allowing the subsequent detection by addition of an enzyme-streptavidin, or an enzyme-avidin complex, followed by the appropriate substrate. Enzymes such as horse-radish peroxidase, alkaline phosphatase, ß-galactosidase or urease are frequently used and these, and other, along with several appropriate substrates are commercially available. Preferred labels with a marker which allows direct detection of the formed complex include the use of a radioactive isotope, such as, eg., iodine, or a lanthanide chelate such as Europium.

In another embodiment designed to detect the presence of a NANBV structural antigen in a body sample from a patient, the sample (e.g. blood, plasma, serum, urine or saliva) is contacted by admixture under biological assay conditions with an anti-NANBV structural protein antibody of this invention, to form an immunoreaction admixture. The admixture is then maintained for a period of time sufficient to allow the formation of a antigen-antibody immunoreaction product containing NANBV structural antigens complexed with an antibody of this invention. The presence and preferably amount, of complex can then be determined, thereby indicating the presence of antigen in the body fluid sample.

In a preferred embodiment, the antibody is present in a solid phase. Still further preferred, the amount of immunocomplex formed is measured by a competition immunoassay format where the antigen in a patient's body fluid sample competes with a labeled recombinant antigen of this invention for binding to the solid phase antibody. The method comprises admixing a body fluid sample with (1) solid support having affixed thereto an antibody according to this invention and (2) a labeled NANBV antigenic molecule of this invention that immunoreacts with the solid phase antibody to form a competition immunoreaction admixture that has both a liquid phase and a solid phase. The admixture is then maintained for a time period sufficient to form a labeled NANBV antigenic molecule-containing immunoreaction product in the solid phase. Thereafter, the amount of label present in the solid phase is determined, thereby indicating the amount of NANBV structural antigen in the body fluid sample.

Enzyme immunoassay techniques, whether direct or competition assays using homogenous or heterogenous assay formats, have been extensively described in the art. Exemplary techniques can be found in Maggio, Enzyme Immunoassay, CRC Press, Cleveland, OH (1981); and Tijssen, "Practice and Theory of Enzyme Immunoassays", Elsevier, Amsterdam (1988).

Biological assay conditions are those that maintain the biological activity of the NANBV antigenic molecules and the anti-NANBV structural protein antibodies in the immunoreaction admixture. Those conditions include a temperature range of about 4°C to about 45°C, preferably about 37°C, a pH value range of about 5 to about 9, preferably about 7, and an ionic strength varying from that of distilled water

to that of about one molar sodium chloride, preferably about that of physiological saline. Methods for optimizing such conditions are well known in the art.

Also contemplated are immunological assays capable of detecting the presence of immunoreaction product formation without the use of a label. Such methods employ a "detection means", which means are themselves well-known in clinical diagnostic chemistry and constitute a part of this invention only insofar as they are utilized with otherwise novel polypeptides, methods and systems. Exemplary detection means include methods known as biosensors and include biosensing methods based on detecting changes in the reflectivity of a surface (surface plasmon resonance), changes in the absorption of an evanescent wave by optical fibers or changes in the propagation of surface acoustical waves.

Another embodiment contemplates detection of the immunoreaction product employing time resolved fluorometry (TR-FIA), where the label used is able to produce a signal detectable by TR-FIA. Typical labels suitable for TR-FIA are metal-complexing agents such as a lanthanide chelate formed by a lanthanide and an aromatic beta-diketone, the lanthanide being bound to the antigen or antibody via an EDTA-analog so that a fluorescent lanthanide complex is formed.

The principle of time-resolved fluorescence is described by Soini et al, Clin. Chem., 25:353-361 (1979), and has been extensively applied to immunoassay. See for example, Halonen et al., Current Topics in Microbiology and Immunology, 104: 133-146 (1985); Suonpaa et al., Clinica Chimica Acta, 145:341-348 (1985): Lovgren et al., Talanta, 31:909-916 (1984); U.S. Patent Nos. 4,374,120 and 4,569,790; and published International Patent Application Nos. EPO

139 675 and W087/02708. A preferred lanthanide for use in TR-FIA is Europium.

Regents and systems for practicing the TR-FIA technology are available through commercial suppliers (Pharmacia Diagnostics, Uppsala, Sweden).

Particularly preferred are the solid phase immunoassays described herein in Example 7, performed as a typical "Western Blot".

The present diagnostic methods may be practiced in combination with other separate methods for detecting the appearance of anti-NANBV antibodies in species infected with NANBV. For example, a composition of this invention may be used together with commercially available C100-3 antigen (Ortho Diagnostics, Inc., Raritan, N.J.) in assays to determine the presence of either or both antibody species immunoreactive with the two antigens.

The present invention also contemplates the use of nucleic acid hybridization methods to detect the presence of NANBV nucleic acids in a body sample using a polynucleotide or DNA segment of this invention. The method generally comprises a) forming an aqueous hybridization admixture by admixing a body sample with a polynucleotide or oligonucleotide of this invention; b) maintaining the aqueous hybridization admixture for a time period and under hybridizing conditions sufficient for any NANBV polynucleic acids present in the body sample to hybridize with the admixed polynucleotides or oligonucleotides to form a hybridization product; and c) detecting the presence of any of the hybridization product formed and thereby the presence of NANBV polynucleic acids in the body sample.

The NANBV nucleic acid sequence to be detected is referred to herein as the target nucleic acid

sequence. Target nucleic acid sequences to be hybridized in the present methods can be present in any nucleic acid-containing sample so long as the sample is in a form, with respect to purity and concentration, compatible with nucleic acid hybridization reaction. Isolation of nucleic acids to a degree suitable for hybridization is generally known and can be accomplished by a variety of means. For instance, nucleic acids can be isolated from a variety of nucleic acid-containing samples including body tissue, such as skin, muscle, hair, and the like, and body fluids such as blood, plasma, urine, amniotic fluids, cerebral spinal fluids, and the like. See, for example, Maniatis et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory (1982); and Ausubel et al., Current Protocols in Molecular Biology, John Wiley and Sons (1987).

The hybridization reaction mixture is maintained in the contemplated method under hybridizing conditions for a time period sufficient for the polynucleotide or oligonucleotide probe to hybridize to complementary nucleic acid sequences present in the sample to form a hybridization product, i.e., a complex containing probe and target nucleic acid.

The phrase "hybridizing conditions" and its grammatical equivalents, when used with a maintenance time period, indicates subjecting the hybridization reaction admixture, in the context of the concentrations of reactants and accompanying reagents in the admixture, to time, temperature and pH conditions sufficient to allow the polynucleotide or oligonucleotide probe to anneal with the target sequence, typically to form a nucleic acid duplex. Such time, temperature and pH conditions required to accomplish hybridization depend, as is well known in

the art, on the length of the polynucleotide or oligonucleotide probe to be hybridized, the degree of complementarity between the polynucleotide or oligonucleotide probe and the target, the guanidine and cytosine content of the polynucleotide or oligonucleotide, the stringency of hybridization desired, and the presence of salts or additional reagents in the hybridization reaction admixture as may affect the kinetics of hybridization. Methods for optimizing hybridization conditions for a given hybridization reaction admixture are well known in the art.

Typical hybridizing conditions include the use of solutions buffered to pH values between 4 and 9, and are carried out at temperatures from 18 degrees C (18°C) to 75°C, preferably about 37°C to about 65°C, more preferably about 54°C, and for time periods from 0.5 seconds to 24 hours, preferably 2 minutes.

Hybridization can be carried out in a homogeneous or heterogeneous format as is well known. The homogeneous hybridization reaction occurs entirely in solution, in which both the polynucleotide probe and the nucleic acid sequences to be hybridized (target) are present in soluble forms in solution. A heterogeneous reaction involves the use of a matrix that is insoluble in the reaction medium to which either the polynucleotide probe or target nucleic acid is bound. For instance, the body sample to be assayed can be affixed to a solid matrix and subjected to in situ hybridization.

In situ hybridization is typically performed on a body sample in the form of a slice or section of tissue usually having a thickness in the range of about 1 micron to about 100 microns, preferably about 1 micron to about 25 microns and more preferably about

1 micron to about 10 microns. Such sample can be prepared using a commercially available cryostat.

Alternatively, a heterogeneous format widely used is the Southern blot procedure in which genomic DNA is electrophoresed after restriction enzyme digestion, and the electrophoresed DNA fragments are first denatured and then transferred to an insoluble matrix. In the blot procedure, a polynucleotide or oligonucleotide probe is then hybridized to the immobilized genomic nucleic acids containing complementary nucleic acid (target) sequences.

Still further, a heterogeneous format widely used is a library screening procedure in which a multitude of colonies, typically plasmid-containing bacteria or lambda bacteriophage-containing bacteria, is plated, cultured and blotted to form a library of cloned nucleic acids on an insoluble matrix. The blotted library is then hybridized with a polynucleotide or oligonucleotide probe to identify the bacterial colony containing the nucleic acid fragments of interest.

Typical heterogeneous hybridization reactions include the use of glass slides, nitro-cellulose sheets, and the like as the solid matrix to which target-containing nucleic acid fragments are affixed.

Also preferred are the homogeneous hybridization reactions such as are conducted for a reverse transcription of isolated mRNA to form cDNA, dideoxy sequencing and other procedures using primer extension reactions in which polynucleotide or oligonucleotide hybridization is a first step. Particularly preferred is the homogeneous hybridization reaction in which a specific nucleic acid sequence is amplified via a polymerase chain reaction (PCR).

Where the nucleic acid containing a target sequence is in a double-stranded (ds) form, it is

preferred to first denature the dsDNA, as by heating or alkali treatment, prior to conducting the hybridization reaction. The denaturation of the dsDNA can be carried out prior to admixture with a polynucleotide or oligonucleotide to be hybridized, or can be carried out after the admixture of the dsDNA with the polynucleotide or oligonucleotide. Where the polynucleotide or oligonucleotide itself is provided as a double-stranded molecule, it too can be denatured prior to admixture in a hybridization reaction mixture, or can be denatured concurrently therewith the target-containing dsDNA.

The method for detecting a specific target nucleic acid sequence is carried out by first conducting the before-described hybridization reaction to form a hybridization product, and then detecting the presence of the formed hybridization product, thereby detecting the presence of the specific nucleic acid sequence in a nucleic acid-containing sample.

A nucleic acid-containing sample can be a body tissue or body fluid, and can be prepared as described before for hybridization reaction admixtures.

The detection of a hybridization product formed in the hybridization reaction can be accomplished by a variety of means. Although there are preferred embodiments disclosed herein for hybridization product detection, it is to be understood that other well known detection means readily apparent to one skilled in the art are suitable for use in the presently contemplated process and associated diagnostic system.

In one approach for detecting the presence of a specific nucleic acid sequence, the polynucleotide or oligonucleotide probe includes a label or indicating group that will render a hybridization product in which the probe is present detectable. Typically such

labels include radioactive atoms, chemically modified nucleotide bases, and the like.

Radioactive elements operatively linked to or present as part of a polynucleotide or oligonucleotide probe provide a useful means to facilitate the detection of a hybridization product. A typical radioactive element is one that produces beta ray emissions. Elements that emit beta rays, such as ^{3}H, ^{14}C, ^{32}P, and ^{35}S represent a class of beta ray emission-producing radioactive element labels. A radioactive polynucleotide or oligonucleotide probe is typically prepared by enzymatic incorporation of radioactively labeled nucleotides into a nucleic acid using DNA polymerase, and then the labeled nucleic acid is denatured to form a radiolabeled polynucleotide or oligonucleotide probe.

Alternatives to radioactively labeled polynucleotide or oligonucleotide probes are polynucleotides or oligonucleotides that are chemically modified to contain metal complexing agents, biotin-containing groups, fluorescent compounds, and the like.

One useful metal complexing agent is a lanthanide chelate formed by a lanthanide and an aromatic beta-diketone, the lanthanide being bound to the nucleic acid, polynucleotide or oligonucleotide via a chelate forming compound such as an EDTA-analogue so that a fluorescent lanthanide complex is formed. See U.S. Patents No. 4,374,120, and No. 4,569,790 and published Patent Applications No. EP0139675 and No. WO87/02708.

Biotin or acridine ester-labeled oligonucleotides and their use in polynucleotides have been described. See U.S. Patent No. 4,707,404, published Patent Application EP0212951 and European Patent No. 0087636.

Useful fluorescent marker compounds include fluorescein, rhodamine, Texas Red, NBD and the like.

A labeled nucleotide present in a hybridization product renders the hybridization product itself labeled and therefore distinguishable over other nucleic acids present in a sample to be assayed. Detecting the presence of the label in the hybridization product and thereby the presence of the hybridization product, typically involves separating the hybridization product from any labeled polynucleotide or oligonucleotide probe that is not hybridized to a hybridization product.

Techniques for the separation of single- stranded polynucleotide or oligonucleotides, such as non-hybridization labeled polynucleotide or oligonucleotide probe, from a hybridized product are well known, and typically involve the separation of single-stranded from non-single- stranded nucleic acids on the basis of their chemical properties. More often separation techniques involve the use of a heterogeneous hybridization format in which the non-hybridized probe is separated, typically by washing, from the hybridization product that is bound to a solid matrix. Exemplary is the Southern blot technique, in which the matrix is a nitrocellulose sheet and the label is ^{32}P. Southern, J. Mol. Biol., 98:503 (1975).

In another embodiment, the hybridization product detection step comprises detecting an amplified nucleic acid product. An amplified nucleic acid product is the product of an amplification process well know in the art that is referred to as the polymerase chain reaction (PCR).

Methods and systems for amplifying a specific nucleic acid sequence are described in U.S. Patents

No. 4,683,195 and No. 4,683,202, both to Mullis et al.; and the teachings in PCR Technology, Erlich, ed., Stockton Press (1989); Faloona et al., Methods in Enzymol., 155:335-50 (1987); and Polymerase Chain Reaction, Erlich et al., eds., Cold Spring Harbor Laboratories Press (1989).

Examples

The following examples are given for illustrative purposes only and do not in any way limit the scope of the invention.

Example 1. Production of Recombinant DNA Molecules

A. Isolation of NANBV Clones and Sequence Analysis

(1) Isolation of NANBV RNA and Preparation of cDNA

As a source for NANB virions, blood was collected from a chimpanzee infected with the Hutchinson (Hutch) strain exhibiting acute phase NANBH. Plasma was clarified by centrifugation and filtration. NANB virions were then isolated from the clarified plasma by immunoaffinity chromatography on a column of NANBV IgG (Hutch strain) coupled to protein G sepharose. NANBV RNA was eluted from the sepharose beads by soaking in guanidinium thiocyanate and the eluted RNA was then concentrated through a cesium chloride (CsCl) cushion. Sambrook et al., Molecular Cloning: A Laboratory Manual, Sambrook et al., eds. Second Edition, Cold Spring Harbor Laboratory Press, NY (1989).

The purified NANBV RNA in picogram amounts was used as a template in a primer extension reaction admixture containing random and oligo dT primers, dNTPs, and reverse transcriptase to form first strand

cDNAs. The resultant first strand cDNAs were used as templates for synthesis of second strand cDNAs in a reaction admixture containing DNA polymerase I and RNAse H to form double stranded (ds) cDNAs (Sambrook et al., supra). The synthesized ds cDNAs were amplified using an asymmetric synthetic primer-adaptor system wherein sense and anti-sense primers were annealed to each other and ligated to the ends of the double stranded NANBV cDNAs with T4 ligase under blunt-end conditions to form cDNA-adaptor molecules. Polymerase chain reaction (PCR) amplification was performed as described below by admixing the cDNA-adaptor molecules with the same positive sense adaptor primers, dNTPs and TAQ polymerase (Promega Biotec, Madison, WI) to prepare amplified NANBV cDNAs. The resultant amplified NANBV cDNA sequences were then used as templates for subsequent amplification in a PCR reaction with specific NANBV oligonucleotide primers.

(2) Synthesis of Oligonucleotides for Use in NANBV Cloning

Oligonucleotides were selected to correspond to the 5' sequence of Hepatitis C which putatively encodes the NANBV structural capsid and envelope proteins (HCJ1 sequence: Okamoto et al., Jap. J. Exp. Med., 60:167-177, 1990). The selected oligonucleotides were synthesized on a Pharmacia Gene Assembler according to the manufacturer's instruction, purified by polyacrylamide gel electrophoresis and have nucleotide base sequences and consecutive SEQ ID NOs beginning with 15 and ending with 23 as shown in Table 1.

TABLE 1

SYNTHETIC OLIGONUCLEOTIDES

Oligonucleotide Designation[a]	Putative NANBV Region	Oligonucleotide Sequence	SEQ ID NO
690 (+)	Capsid 1-21	ATGAGCACGATTCCCAAACCT	15
693 (+)	Capsid 146-162	GAGGAAGACTTCCGAGC	16
694 (-)	Capsid 208-224	GTCCTGCCCTCGGGCCG	17
691 (-)	Capsid 340-359	ACCCAAATTGCGCGACCTACG	18
14 (+)	Envelope 356-374	TGGGTAAGGTCATCGATAC	19
15 (+)	Envelope 361-377	AAGGTCATCGATACCCT	20
18 (-)	Envelope 512-529	AGATAGAGAAAGAGCAAC	21
16 (-)	Envelope 960-981	GGACCAGTTCATCATCATATAT	22
17 (-)	Envelope 957-976	CAGTTCATCATCATATCCCA	23

a The oligonucleotides are numerically defined and their polarity is indicated as (+) and (-) indicating the sequence corresponds to the sense and anti-sense coding strand, respectively. All sequences are listed in the 5' to 3' orientation.

(3) PCR Amplification of NANBV cDNA

PCR amplification was performed by admixing the primer-adapted amplified cDNA sequences prepared in Example 1A(1) with the synthetic oligonucleotides 690 and 694 as primer (primer pairs 690:694). The resulting PCR reaction admixture contained the primer-adapted amplified cDNA template, oligonucleotides 690 and 694, dNTPs, salts (KCl and $MgCl_2$) and TAQ polymerase. PCR amplification of the cDNA was conducted by maintaining the admixture at a 37°C annealing temperature for 30 cycles. Aliquots of samples from the first round of amplification were reamplified at a 55°C annealing temperature for 30 cycles under similar conditions.

(4) Preparation of Vectors Containing PCR Amplified ds DNA

Aliquots from the second round of PCR amplification were subjected to electrophoresis on a 5% acrylamide gel. After separation of the PCR reaction products, the region of the gel containing DNA fragments corresponding to the expected 690:694 amplified product of approximately 224 bp was excised and purified following standard electroelution techniques (Sambrook et al., supra). The purified fragments were kinased and cloned into the pUC18 plasmid cloning vector at the Sma I polylinker site to form a plasmid containing the DNA segment 690:694 operatively linked to pUC18.

The resulting mixture containing pUC18 and a DNA segment corresponding to the 690:694 sequence region was then transformed into the E. coli strain JM83. Plasmids containing inserts were identified as lac$^-$ (white) colonies on X-gal medium containing ampicillin. pUC18 plasmids which contained the 690:694 DNA segment were identified by restriction enzyme analysis and subsequent electrophoresis on agarose gels, and were designated pUC18 690:694 rDNA molecules.

(5) Sequencing of Hepatitis Clones that Encode the Putative Capsid Protein

Two independent colonies believed to contain a pUC18 vector having the NANBV Hutch strain 690:694 DNA segment (pUC18 690:694) that codes for the amino terminus of the putative capsid protein were amplified and used to prepare plasmid DNA by CsCl density gradient centrifugation by standard procedures (Sambrook et al., supra). The plasmids were sequenced using ^{35}S dideoxy procedures with pUC 18 specific primers. The two plasmids were independently

sequenced on both DNA strands to assure the accuracy of the sequence. The resulting sequence information is presented as base 1 to base 224 of SEQ ID NO:1.

Plasmid pUC18 690:694 contains a NANBV DNA segment that is 224 bp in length and when compared to the HCJ1 prototype sequence reveals two nucleotide substitutions and one amino acid residue difference in the amino terminal region of the putative capsid protein.

(6) Preparation of NANBV Clones from the 5' End of the Genome

To obtain the sequence of the NANBV Hutch genome encoding the remainder of the capsid region (Okamoto et al., supra), the oligonucleotides 693 and 691 (described in Table 1) were used in PCR reactions. cDNA was prepared as described in Example 1A(1) to viral NANBV RNA from Hutch and used in PCR amplification as described in Example 1A(3) with the oligonucleotide pair 693:691. The resultant PCR amplified ds DNA was then cloned into pUC18 cloning vectors and screened for inserts as described in Example 1A(4) to form pUC 18 693:691. Clones were then sequenced with pUC18 specific primers as described in Example 1A(5).

Plasmid pUC18 693:691 contains a NANBV DNA segment that is 157 bp in length and spans nucleotide bases 203 to 360 of SEQ ID NO:1. The segment does not extend to the sequence of the 693 primer used for generating the fragment. The sequence of this fragment reveals three nucleotide differences when compared to the known sequence of HCJ1 and does not have any corresponding amino acid changes to the HCJ1 sequence.

To obtain the sequence of the NANBV Hutch genome encoding the putative envelope region (Okamoto et al.,

supra), the oligonucleotide primers 14 through 18 (described in Table 1) were used in various combinations with NANBV Hutch RNA samples. As a source of NANBV RNA, a liver biopsy specimen from a chimpanzee inoculated with the Hutch strain at 4 weeks post-inoculation and exhibiting acute infection was used. The biopsied sample was first frozen and then ground. The resultant powder was the treated with guanidine isothiocyanate for the extraction of RNA. RNA was extracted from the guanidium-treated liver samples with phenol in the presence of SDS at 65°C. The liver samples were extracted a second time, and then extracted with chloroform. The extracted RNA was precipitated at -20°C with isopropanol and sodium acetate.

The purified liver-derived RNA was used as a template in primer extension reactions with the oligonucleotides 18 and 16 to generate NANBV specific-cDNAs. To prepare cDNA to the Hutch strain amino-terminal protein coding sequences, anti-sense oligonucleotides, 18 and 16, were annealed to liver-derived Hutch RNA in the presence of dNTPs and reverse transcriptase at 42°C to form primer extension products. The first round of PCR amplification of the two cDNAs was performed by admixing the primer extension reaction products with separate pairs of oligonucleotides 14:16 (16 primed cDNA) and 14:18 (18 primed cDNA) for 30 cycles at 55°C annealing temperature. The PCR reactions were performed on the above admixture as in 1A(3). Aliquots from the 14:16 and 14:18 amplifications were used as templates for the second round of amplification in which the oligonucleotide pairs 15:17 and 15:18, respectively, were used as primers.

PCR reaction products from each of the primer pair reactions were analyzed by electrophoresis on low melt agarose gels. Following separation, the regions of the gel containing DNA fragments corresponding to the expected 15:17 and 15:18 amplified products of approximately 617 bp and 168 bp, respectively, were excised and eluted from the gel slices at 65°C. The resultant eluted fragments were purified by phenol and chloroform extractions. To clone the 15:17 and 15:18 fragments, the purified fragments were separately treated with the Klenow fragment of DNA polymerase and kinase for subsequent subcloning into the SmaI site of the pBluescript plasmid vector (Stratagene Cloning Systems, La Jolla, CA). Transformed E. coli DH5 colonies were analyzed for plasmid insert by restriction enzyme analysis as described in Example 1A(4).

pBluescript plasmid containing 15:17 or 15:18 DNA segments were purified using large scale CsCl plasmid preparation protocols. The DNA segments present in the amplified and purified plasmids were each sequenced as described in Example 1A(5).

The sequence of the 15:17 DNA segment is contained in SEQ ID NO:1 from nucleotide 361 to 978. The sequence of the 15:18 DNA segment is also presented in SEQ ID NO:1 from nucleotide 361 to 529. These two clones overlap by 168 bp of the 15:18 DNA segment.

The sequence results indicate that the 15:17 DNA segment differs by 30 nucleotides when compared to the HCJ1 sequence (Okamoto et al., supra) and also differs by ten amino acid residues. The 15:18 DNA segment differs by seven nucleotides and by three amino acid residues when compared to HCJ1. In the overlap region, the two DNA segments differ at two nucleotide

bases, namely, bases 510 and 511, where DNA segment 15:18 contains a C in place of a T and an A in place of a G, respectively, which results in a change of a serine in place of a glycine amino acid residue, at residue 171 of SEQ ID NO:1. The reason for these differences is unknown and may be due to a PCR artifact.

B. Production of Recombinant DNA (rDNA) that Encodes a Fusion Protein

(1) Isolation of the 690:694 Fragment from the pUC 18 Clone and Introduction of the Fragment into the pGEX-3X Expression Vector

The pUC18 vector containing the 690:694 DNA segment was subjected to restriction enzyme digestion with Eco RI and Bam HI to release the DNA segment that includes a sequence contained in SEQ ID NO:1 from base 1 to base 224 from the pUC18 vector. The released DNA segment was subjected to acrylamide gel electrophoresis and the DNA segment containing the 224 bp NANBV insert plus portions of the pUC 18 polylinker was then excised and eluted from the gel as described in Example 1A(4). The eluted DNA segment was extracted with a mixture of phenol and chloroform, and precipitated.

The precipitated DNA segment was resuspended to a concentration of 25 μg/ml in water and treated with the Klenow fragment of DNA polymerase I and dNTP to fill in the staggered ends created by the restriction digestion. The resultant blunt-ended 690:694 segment was admixed with the bacterial expression vector, pGEX-3X, (available from Pharmacia Inc., Piscataway, NJ) which was linearized with the blunt end restriction enzyme Sma I. The admixed DNAs were then covalently linked (ligated) by maintaining the admixture overnight at 16°C in the presence of ligase

buffer and 5 units of T4 DNA ligase to form a plasmid of 690:694 DNA segment operatively linked to pGEX-3X.

(2) Selection and Verification of Correctly Oriented Ligated Insert

The ligation mixture containing the pGEX-3X vector and the 690:694 DNA segment was transformed into host E. coli strain W3110. Plasmids containing inserts were identified by selection of host bacteria containing vector in Luria broth (LB) media containing ampicillin. Bacterial cultures at stationary phase were subjected to alkaline lysis protocols to form a crude DNA preparation. The DNA was digested with the restriction enzyme Xho I. The single Xho I site, which cleaves within the 690:694 DNA segment between nucleotide positions 173 to 178 of SEQ ID NO:1, but not within the pGEX-3X vector, was used to screen for vector containing the 690:694 DNA segment.

Several 690:694 DNA segment-containing vectors were amplified and the resultant amplified vector DNA was purified by CsCl density gradient centrifugation. The DNA was sequenced across the inserted DNA segment ligation junctions by ^{35}S dideoxy methods with a primer that hybridized to the pGEX-3X sequence at nucleotide positions 614 to 633 contained in SEQ ID NO:2. Vectors containing 690:694 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein were thus identified and selected to form pGEX-3X-690:694.

(3) Structure of the Fusion Protein

The pGEX-3X vector is constructed to allow for inserts to be placed at the C terminus of Sj26, a 26-kDa glutathione S-transferase (GST; EC 2.5.1.18) encoded by the parasitic helminth Schistosoma japonicum. Insertion of the 690:694 NANBV fragment in-frame behind Sj26 allows for the synthesis of the

Sj26-NANBV fusion polypeptide. The NANBV polypeptide can be cleaved from the GST carrier by digestion with the site-specific protease factor Xa (Smith et al., Gene, 67:31-40, 1988).

The nucleotide and predicted amino acid sequence of the pGEX-3X-690:694 fusion transcript from the GST sequence through the 690:694 insert is presented in SEQ ID NO:2. The resulting rDNA molecule, pGEX-3X-690:694, is predicted to encode a NANBV fusion protein having the amino acid residue sequence contained in SEQ ID NO:2 from amino acid residue 1 to residue 315. The resulting protein product generated from the expression of the plasmid is referred to as both the GST:NANBV 690:694 fusion protein and the CAP-N fusion protein.

C. Production of Recombinant DNAs (rDNAs) that Encode NANBV Capsid and Envelope Fusion Proteins

pGEX-3X-693:691: Plasmid pGEX-3X-693:691 was formed by first subjecting the plasmid pUC 18 693:691 prepared in Example 1A(6) to restriction enzyme digestion with Eco RI and Bam HI as performed in Example 1B(1). The resultant released DNA segment having a sequence contained in SEQ ID NO:1 from base 205 to base 360 was purified as performed in Example 1B(1). The purified DNA segment was admixed with and ligated to the pGEX-3X vector which was linearized by restriction enzyme digestion with Eco RI and Bam HI in the presence of T_4 ligase at 16°C to form the plasmid pGEX-3X-693:691.

A pGEX-3X plasmid containing a 693:691 DNA segment was identified by selection as performed in Example 1B(2) with the exception that crude DNA preparations were digested with Eco RI and Bam HI to release the 693:691 insert. A pGEX-3X vector

containing a 693:691 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein was identified by sequence analysis as performed in Example 1B(2) and selected to form pGEX-3X-693:691.

The resulting vector encodes a fusion protein (GST:NANBV 693:691) that is comprised of an amino-terminal polypeptide portion corresponding to residues 1 to 221 of GST as contained in SEQ ID NO:2, an intermediate polypeptide portion corresponding to residues 222 to 225 and defining a cleavage site for the protease Factor Xa, a linker protein corresponding to residues 226 to 230 consisting of the amino acid residue sequence (SEQ ID NO:25):

Gly Ile Pro Asn Ser

encoded by the nucleotide base sequence (SEQ ID NO:24):

GGG ATC CCC AAT TCA, respectively;

a carboxy-terminal polypeptide portion corresponding to residues 231 to 282 defining a NANBV capsid antigen having the amino acid residue sequence 69 to 120 in SEQ ID NO:1, and a carboxy-terminal linker portion corresponding to residues 283 to 287 consisting of the amino acid residue sequence (SEQ ID NO:27):

Asn Ser Ser END

encoded by the nucleotide base sequence (SEQ ID NO:26):

AAT TCA TCG TGA, respectively.

pGEX-3X-15:18: Plasmid pGEX-3X-15:18 was formed by first subjecting the plasmid Bluescript 15:18 prepared in Example 1A(6) to restriction enzyme digestion with Eco RV and Bam HI and the Bam HI cohesive termini were filled in as performed in Example 1B(1). The resultant released DNA segment having a sequence contained in SEQ ID NO:1 from base

361 to base 528 was purified as performed in Example 1B(1). The purified DNA segment was admixed with and ligated to the pGEX-3X vector which was linearized by restriction enzyme digestion with Sma I as performed in 1B(1) to form the plasmid pGEX-3X-15:18.

A pGEX-3X plasmid containing a 15:18 DNA segment was identified by selection as performed in Example 1B(2) and crude DNA preparations were cut with Eco RI and Bam HI to release the 15:18 inserts. A pGEX-3X vector containing a 15:18 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein was identified as performed in Example 1B(2) and selected to form pGEX-3X-15:18.

The resulting vector encodes a fusion protein (GST:NANBV 15:18) that is comprised of an amino-terminal polypeptide portion corresponding to residues 1 to 221 of GST, an intermediate polypeptide portion corresponding to residues 222 to 225 and defining a cleavage site for the protease Factor Xa, a linker protein corresponding to residues 226 to 234 consisting of the amino acid residue sequence (SEQ ID NO:29):

Gly Ile Pro Ile Glu Phe Leu Gln Pro,

encoded by the nucleotide base sequence (SEQ ID NO:28):

GGG ATC CCC ATC GAA TTC CTG CAG CCC,

respectively; a carboxy-terminal polypeptide portion corresponding to residues 235 to 290 defining a NANBV envelope antigen having the amino acid residue sequence 121 to 176 in SEQ ID NO:1, and a carboxy-terminal linker portion corresponding to residues 291 to 298 consisting of a amino acid residue sequence (SEQ ID NO:31):

Trp Gly Ile Gly Asn Ser Ser END

encoded by the nucleotide base sequence (SEQ ID NO:30):

TGG GGG ATC GGG AAT TCA TCG TGA, respectively.

pGEX-3X-15:17: Plasmid pGEX-3X-15:17 was formed by first subjecting the plasmid Bluescript 15:17 prepared in Example 1A(6) to restriction enzyme digestion with Eco RI and Bam HI and the cohesive termini were filled in as performed in Example 1B(1). The resultant released DNA segment having a sequence contained in SEQ ID NO:1 from base 361 to base 978 was purified as performed in Example 1B(1). The purified DNA segment was admixed with and ligated to the pGEX-3X vector which was linearized by restriction enzyme digestion with Sma I as performed in Example 1B(1) to form the plasmid pGEX-3X-15:17.

A pGEX-3X plasmid containing a 15:17 DNA segment was identified by selection as performed in Example 1B(2) and DNA preparations were digested with Eco RI and Bam HI as indicated above. pGEX-3X vector containing a 15:17 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein was identified as performed in Example 1B(2) and selected to form pGEX-3X-15:17.

The resulting vector encodes a fusion protein (GST:NANBV 15:17) that is comprised of an amino-terminal polypeptide portion corresponding to residues 1 to 221 of GST, an intermediate polypeptide portion corresponding to residues 222 to 225 and defining a cleavage site for the protease Factor Xa, a linker protein corresponding to residues 226 to 233 consisting of the amino acid residue sequence (SEQ ID NO:33):

Gly Ile Pro Asn Ser Cys Ser Pro

encoded by the nucleotide base sequence (SEQ ID NO:32):

GGG ATC CCC AAT TCC TGC AGC CCT, respectively; a carboxy-terminal polypeptide portion corresponding to residues 234 to 439 defining a NANBV envelope antigen having the amino acid residue sequence 121 to 326 in SEQ ID NO:1, and a carboxy-terminal linker portion corresponding to residues 440 to 446 consisting of the amino acid residue sequence (SEQ ID NO:35):

Gly Ile Gly Asn Ser Ser END

encoded by the nucleotide base sequence (SEQ ID NO:34):

GGG ATC GGG AAT TCA TCG TGA, respectively.

pGEX-2T-15:17: Plasmid pGEX-2T-15:17 was formed by first subjecting the plasmid Bluescript 15:17 prepared in Example 1A(6) to restriction enzyme digestion with Eco RV and Bam HI and the Bam HI cohesive termini were filled in as performed in Example 1B(1). The resultant released DNA segment having a sequence contained in SEQ ID NO:1 from base 361 to base 978 was purified as performed in Example 1B(1). The purified DNA segment was admixed with and ligated to the pGEX-2T vector (Pharmacia, INC.) which was linearized by restriction enzyme digestion with Sma I as performed in Example 1B(1) to form the plasmid pGEX-2T-15:17.

A pGEX-2T plasmid containing a 15:17 DNA segment was identified by selection as performed in Example 1B(2) and by digestion of crude DNA preparations with Eco RI and Bam HI. A pGEX-2T vector containing a 15:17 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein was identified as performed in Example 1B(2) and selected to form pGEX-2T-15:17.

The resulting vector encodes a fusion protein (GST:NANBV 15:17) that is comprised of an amino-terminal polypeptide portion corresponding to

residues 1 to 221 of GST, an intermediate polypeptide portion corresponding to residues 222 to 226 and defining a cleavage site for the protease Thrombin consisting of the amino acid residue sequence (SEQ ID NO:37):

Val Pro Arg Gly Ser

encoded by the nucleotide base sequence (SEQ ID NO:36):

GTT CCG CGT GGA TCC, respectively;

a linker protein corresponding to residues 227 to 233 consisting of an amino acid residue sequence (SEQ ID NO:39):

Pro Ser Asn Ser Cys Ser Pro

encoded by a nucleotide base sequence (SEQ ID NO:38):

CCA TCG AAT TCC TGC AGC CCT,

respectively; a carboxy-terminal polypeptide portion corresponding to residues 234 to 439 defining a NANBV envelope antigen, and a carboxy-terminal linker portion corresponding to residues 440 to 446 consisting of the amino acid residue sequence (SEQ ID NO:41):

Gly Ile His Arg Asp END

encoded by the nucleotide base sequence (SEQ ID NO:40):

GGA ATT CAT CGT GAC TGA, respectively.

pGEX-3X-690:691: To obtain a DNA segment corresponding to the NANBV Hutch sequence shown from SEQ ID NO:1 from base 1 to base 360, the oligonucleotides 690:691 are used in PCR reactions as performed in Example 1A(6). The resultant PCR amplified ds DNA is then cloned into pUC 18 cloning vectors as described in Example 1A(4) to form pUC18 690:691. Clones are then sequenced with pUC18 primers as described in Example 1A(5) to identify a plasmid containing the complete sequence. The resulting

identified plasmid is selected, is designated pUC18 690:691, and contains a NANBV DNA segment that is 361 bp in length and spans nucleotides 1 to 360 of SEQ ID NO:1.

Plasmid pGEX-3X-690:691 is formed by first subjecting the plasmid pUC18 690:691 to restriction enzyme digestion with Eco RI and Bam HI as performed in Example 1B(1). The resultant released DNA segment having a sequence contained in SEQ ID NO:1 from base 1 to base 360 with pUC18 polylinker sequence is purified as performed in Example 1B(1). The purified DNA segment is admixed with and ligated to the pGEX-3X vector which is linearized by restriction enzyme digestion with Sma I as performed in Example 1B(1) to form the plasmid pGEX-3X-690:691.

A pGEX-3X plasmid containing a 690:691 DNA segment is identified by selection as performed in Example 1B(2). pGEX-3X vector containing a 690:691 DNA segment having the correct coding sequence for in-frame translation of a NANBV structural protein is identified as performed in Example 1B(2) and selected to form pGEX-3X-690:691.

The resulting vector encodes a fusion protein (GST:NANBV 690:691) that is comprised of an amino-terminal polypeptide portion corresponding to residues 1 to 221 of GST, an intermediate polypeptide portion corresponding to residues 222 to 225 and defining a cleavage site for the protease Factor Xa, a linker protein corresponding to residues 226 to 234 consisting of the amino acid residue sequence (SEQ ID NO:43):

Gly Ile Pro Asn Ser Ser Ser Val Pro

encoded by the nucleotide base sequence (SEQ ID NO:42):

GGG ATC CCC AAT TCG AGC TCG GTA CCC

respectively; a carboxy-terminal polypeptide portion corresponding to residues 235 to 355 defining a NANBV capsid antigen, and a carboxy-terminal linker portion corresponding to residues 356 to 363 consisting of the amino acid residue sequence (SEQ ID NO:45):

Thr Gly Ile Gly Asn Ser Ser END

encoded by the nucleotide base sequence (SEQ ID NO:44):

ACG GGG ATC GGG AAT TCA TCG TGA, respectively.

pGEX-2T-CAP-A: Oligonucleotides 1-20(+) and 1-20(-) for constructing the vector pGEX-2T-CAP-A for expressing the CAP-A fusion protein were prepared as described in Example 1A(2) having nucleotide base sequences corresponding to SEQ ID NO:7 and SEQ ID NO:8, respectively.

Oligonucleotides 1-20 (+) and 1-20 (-) were admixed in equal amounts with the expression vector pGEX-2T (Pharmacia) that had been predigested with Eco RI and Bam HI and maintained under annealing conditions to allow hybridization of the complementary oligonucleotides and to allow the cohesive termini of the resulting double-stranded (ds) oligonucleotide product to hybridize with pGEX-2T at the Eco RI and Bam HI cohesive termini. After ligation the resulting plasmid designated pGEX-2T-CAP-A contains a single copy of the ds oligonucleotide product and a structural gene coding for a fusion protein designated CAP-A having an amino acid residue sequence shown in SEQ ID NO:3 from residue 1 to residue 252.

The pGEX-2T vector is similar to the pGEX-3X vector described above, except that the resulting fusion protein is cleavable by digestion with the site specific protease thrombin.

pGEX-2T-CAP-B: Oligonucleotides 21-40(+) and 21-40(-) for constructing the vector pGEX-2T-CAP-B for

expressing the CAP-B fusion protein were prepared as described in Example 1A(2) having nucleotide base sequences corresponding to SEQ ID NO:9 and SEQ ID NO:10, respectively.

Oligonucleotides 21-40 (+) and 21-40 (-) were admixed in equal amounts with the pGEX-2T expression vector that had been predigested with Eco RI and Bam HI and maintained under annealing conditions to allow hybridization of the complementary oligonucleotides and to allow the cohesive termini of the resulting double-stranded oligonucleotide product to hybridize with pGEX-2T at the Eco RI and Bam HI cohesive termini. After ligation the resulting plasmid designated as pGEX-2T-CAP-B contains a single copy of the ds oligonucleotide product and contains a structural gene coding for a fusion protein designated CAP-B having an amino acid residue sequence shown in SEQ ID NO:4 from residue 1 to residue 252.

pGEX-2T-CAP C: Oligonucleotides 41-60(+) and 41-60(-) for constructing the vector pGEX-2T-CAP-C for expressing the CAP-C fusion protein were prepared as described in Example 1A(2) having nucleotide base sequences corresponding to SEQ ID NO:11 and SEQ ID NO:12, respectively.

Oligonucleotides 41-60 (+) and 41-60 (-) were admixed in equal amounts with the pGEX-2T expression vector that had been predigested with Eco RI and Bam HI and maintained under annealing conditions to allow hybridization of the complementary oligonucleotides and to allow the cohesive termini of the resulting double-stranded oligonucleotide product to hybridize with pGEX-2T at the Eco RI and Bam HI cohesive termini. After ligation the resulting plasmid designated as pGEX-2T-CAP-C contains a single copy of the double-stranded oligonucleotide product and

contains a structural gene coding for a fusion protein designated CAP-C having an amino acid residue sequence shown in SEQ ID NO:5 from residue 1 to residue 252.

pGEX-2T-CAP-A-B: Oligonucleotides for constructing the vector pGEX-2T-CAP-A-B for expressing the CAP-A-B fusion protein were prepared as described in Example 1A(2) having nucleotide base sequences corresponding to SEQ ID NO:13 and SEQ ID NO:14, respectively.

Oligonucleotides according to SEQ ID NO:13 and SEQ ID NO:14 were admixed in equimolar amounts with the plasmid pGEX-3X-690:694 described in Example 1B(2). The admixture was combined with the reagents for a polymerase chain reaction (PCR) and the two admixed oligonucleotides were used as primers on the admixed pGEX-3X-690:694 as template in a PCR reaction to form a PCR extension product consisting of a double-stranded nucleic acid molecule that encodes the amino acid residue sequence contained in SEQ ID NO:1 from residue 2 to 40 and also includes PCR-added restriction sites for Bam HI at the 5' terminus and Eco RI at the 3' terminus. The PCR extension product was then cleaved with the restriction enzymes Bam HI and Eco RI to produce cohesive termini on the PCR extension product. The resulting product with cohesive termini was admixed in equal amounts with the pGEX-2T expression vector that had been predigested with Eco RI and Bam HI and maintained under annealing conditions to allow the cohesive termini of the double-stranded PCR extension product to hybridize with pGEX-2T at the Eco RI and Bam HI cohesive termini. After ligation the resulting plasmid designated pGEX-2T-CAP-A-B contains a single copy of the double-stranded PCR extension product and contains a structural gene coding for a fusion protein

designated CAP-A-B having an amino acid residue sequence shown in SEQ ID NO:6 from residue 1 to residue 271.

Example 2. Expression of the NANBV 690:694 Fusion Protein Using rDNA

The bacterial colonies which contain the pGEX-3X-690:694 plasmid in the correct orientation were selected to examine the properties of the fusion protein. Bacterial cultures of pGEX-3X-690:694 were grown to a stationary phase in the presence of ampicillin (50 μg/ml final concentration) at 37°C. This culture was inoculated at a 1:50 dilution into fresh LB medium at 37°C in the presence of ampicillin and maintained at 37°C with agitation at 250 rpm until the bacteria reached an optical density of 0.5 when measured using a spectrometer with a 550 nm wavelength light source detector. Isopropylthio-beta-D-galactoside (IPTG) was then admixed to the bacterial culture at a final concentration of 1 mM to initiate (induce) the synthesis of the fusion protein under the control of the tac promoter in the pGEX-3X vector.

Beginning at zero time and at one hour intervals thereafter for three hours following admixture with IPTG (i.e., the induction phase), the bacterial culture was maintained as above to allow expression of recombinant protein. During this maintenance phase, the optical density of the bacterial culture was measured and 1 ml aliquots were removed for centrifugation. Each resultant cell pellet containing crude protein lysate was resuspended in Laemmli dye mix containing 1% beta-mercaptoethanol at a final volume of 50 μl for each 0.5 OD 550 unit. Samples

were boiled for 15 minutes and 10 μl of each sample was electrophoresed on a 10% SDS-PAGE Laemmli gel.

Other GST:NANBV fusion proteins were also expressed in bacteria by transformation with the appropriate expression vector and induction as described above.

Example 3. Detection of Expressed Fusion Proteins

To visualize the IPTG-induced fusion proteins, the Laemmli gels were stained with Coomassie Blue and destained in acetic acid and methanol. Induced proteins from separate clones were examined and compared on the basis of the increase of a protein band in the predicted size range from time zero to time three hours post-IPTG treatment. Expression of fusion protein was observed in clones that exhibited an increase from zero time in the intensity of a protein band corresponding to the fusion protein.

The GST:NANBV fusion proteins CAP-A, CAP-B, and CAP-C, when analyzed on a 12.5% PAGE Laemmli gel as described in Example 2, exhibited an apparent molecular weight of about 30,000 daltons.

Example 4. Western Blot Analysis

Samples from IPTG inductions containing a GST:NANBV fusion protein of this invention were separated by gel electrophoresis and were transferred onto nitrocellulose for subsequent immunoblotting analysis. The nitrocellulose filter was admixed with antibody blocking buffer (20 mM sodium phosphate, pH 7.5, 0.5 M sodium chloride, 1% bovine serum albumin, and 0.05% Tween 40) for 3 to 12 hours at room temperature. Sera from humans or chimpanzees with NANB hepatitis believed to contain antibody immunoreactive with NANBV structural protein was

diluted 1:500 in the antibody blocking buffer and admixed with the nitrocellulose and maintained for 12 hours at room temperature to allow the formation of an immunoreaction product on the solid phase. The nitrocellulose was then washed three times in excess volumes of antibody blocking buffer. The washes were followed by admixture of the nitrocellulose with 50 µl of ^{125}I protein A (New England Nuclear, Boston, MA) at a 1:500 dilution in antibody blocking buffer for one hour at room temperature to allow the labeled protein A to bind to any immunoreaction product present in the solid phase on the nitrocellulose. The nitrocellulose was then washed as described herein, dried and exposed to X-ray film for one to three hours at -70°C in order to visualize the label and therefore any immunoreaction product on the nitrocellulose.

Results of the Western blot immunoassay are shown in Tables 2 through 7. Samples prepared using pGEX-3X vector that produces control GST were also prepared as above and tested using the Western blot procedure as a control. The expressed GST protein was not detectable as measured by immunoreactivity using the sera shown to immunoreact with a fusion protein of this invention (e.g., GST:NANBV 690:694 fusion protein).

Example 5. Purification of Expressed GST:NANBV Fusion Proteins

Cultures of E. coli strain W3110 transformed with recombinant pGEX-3X-690:694 plasmids prepared in Example 2 were cultured for 3 hours following IPTG induction treatment. The cells were then centrifuged to form a bacterial cell pellet, the cells were resuspended in 1/200 culture volume in lysis buffer (MTPBS: 150 mM NaCl, 16 mM Na_2HPO_4, 4 mM NaH_2PO_4, pH 7.3), and the cell suspension was lysed with a French

pressure cell. Triton X-100 was admixed to the cell lysate to produce a final concentration of 1%. The admixture was centrifuged at 50,000 X g for 30 minutes at 4°C. The resultant supernatant was collected and admixed with 2 ml of 50% (w/v) glutathione agarose beads (Sigma, St. Louis, MO) preswollen in MTPBS. After maintaining the admixture for 5 minutes at 25°C to allow specific affinity binding between GST and glutathione in the solid phase, the beads were collected by centrifugation at 1000 X g and washed in MTPBS three times.

The GST:NANBV 690:694 fusion protein was eluted from the washed glutathione beads by admixture and incubation of the glutathione beads with 2 ml of 50 mM Tris HCl, pH 8.0, containing 5 mM reduced glutathione for 2 minutes at 25°C to form purified GST:NANBV 690:694 fusion protein.

The above affinity purification procedure produced greater than 95% pure fusion protein as determined by SDS PAGE. That is, the purified protein was essentially free of procaryotic antigen and non-structural NANBV antigens as defined herein.

Alternatively, GST:NANBV 690:694 fusion protein was purified by anion exchange chromatography. Cultures were prepared as described above and cell pellets were resuspended in 8M guanidine and maintained overnight at 4°C to solubilize the fusion protein. The cell suspension was then applied to an S-300 sepharose chromatography column and peak fractions containing the GST:NANBV 690:694 fusion protein were collected, pooled, dialyzed in 4 M urea and subjected to anion exchange chromatography to form purified fusion protein.

Other GST:NANBV fusion proteins described herein were also expressed in cultures of E.coli Strain W3110

as described above using the GST fusion protein vectors produced in Example 1 after their introduction by transformation into the E.coli host. After induction and lysis of the cultures, the GST fusion proteins were purified as described above using glutathione agarose affinity chromatography to yield greater than 95% pure fusion protein as determined by SDS-PAGE. Thus, CAP-A, CAP-B and CAP-C fusion proteins were all expressed and purified as above using the pGEX-2T-CAP-A vector, the pGEX-2T-CAP-B vector, or the pGEX-2T-CAP-C vector, respectively, and CAP-A-B fusion protein is expressed and purified using the PGEX-2T-CAP-A-B vector.

Example 6. Protease Cleavage of Purified GST:NANBV 690:694 Fusion Protein

Purified GST:NANBV 690:694 fusion protein prepared in Example 5 is subjected to treatment with activated Factor (Xa) (Sigma) to cleave the GST carrier from the NANBV 690:694 fusion protein (Smith et al., supra). Seven μg of Factor X are activated prior to admixture with purified fusion proteins by admixture and maintenance with 75 nanograms (ng) activation enzyme, 8 mM Tris-HCl (pH 8.0), 70 mM NaCl and 8 mM $CaCl_2$ at 37°C for 5 minutes. Fifty μg of purified fusion protein are then admixed with 500 ng activated human factor Xa in the elution buffer described in Example 5 containing 50 mM Tris HCl, 5 mM reduced glutathione, 100 mM NaCl, and 1 mM $CaCl_2$, and maintained at 25°C for 30 minutes. The resulting cleavage reaction products are then absorbed on glutathione-agarose beads prepared in Example 5 to affinity bind and separate free GST from any cleaved NANBV structural antigen-containing protein. Thereafter the liquid phase is collected to form a

solution containing purified NANBV structural protein having an amino acid residue sequence contained in SEQ ID NO:2 from residue 226 to residue 315.

Example 7. Immunological Detection of Anti-NANBV Structural Protein Antibodies

NANBV Hutch strain virus was injected in chimpanzees and blood samples were collected at various weekly intervals post to inoculation (INOC) to analyze the immunological response to NANBV by five different diagnostic assays. Chimpanzees were categorized as either being in the acute or chronic phase of infection. The assays utilized in the evaluation of the immune response include: 1) alanine aminotransferase (ALT) enzyme detection (Alter et al., JAMA, 246:630-634, 1981; and Aach et al., N. Engl. J. Med., 304:989-994, 1981); 2) histological evaluation for NANBV virions by electron microscopy (EM); 3) detection of anti-HCV antibodies using the commercially available kit containing C100-3 antigen (Ortho Diagnostics, Inc.); 4) detection of anti-CAP-N antibodies by immunoblot analysis as described in Example 4 using the CAP-N fusion protein; and 5) Detection of virus by PCR amplification as described in Example 1.

In Table 2, results are presented from ALT, EM, anti-HCV (anti-C100-3), anti-CAP-N, and PCR assays on sera from a chimpanzee with acute NANB Hepatitis.

TABLE 2

CHIMP 59 - ACUTE NANB HEPATITIS

WEEK POST INOC[1]	ALT	EM	ANTI HCV	ANTI CAP-N[2]	PCR 690-691
8	26	++	-	-	-
10	26	+	-	+	-
12	107	+	-	+	-
14	115	+	+	+	-
16	26	+	+	+	+
18	17	ND	+	+	(+)
20	11	ND	+	+	-

[1] Week after inoculation.

[2] A plus (+) indicates immunoreaction was observed between admixed serum and the fusion protein, designated "CAP-N" because it corresponds to the amino terminal of the putative NANBV capsid protein, using the Western blot immunoassay described in Example 4.

The results in Table 2 show immunoreaction between fusion protein and anti-NANBV structural protein antibodies in the sera tested. Furthermore, seroconversion is detectable by the immunoassay using fusion protein containing capsid antigen at times earlier than when the same sera is assayed in the C100-3-based immunoassay.

In Table 3, results are presented from ALT, anti-HCV (anti-C100-3) and anti-CAP-N assays on sera collected from a human with definitive NANB Hepatitis.

TABLE 3

NYU - 169 - DEFINITIVE NANB HEPATITIS

Week Post Infect	ALT	Anti HCV	Anti CAP-N
2	34	-	-
6	8	-	-
10	150	-	-
12	118	-	-
14	183	-	+
16	317	-	+
19	213	-	+
23	53	-	+

The results in Table 3 show that in the human series 169 seroconversion sera samples, the CAP-N antigen present in the fusion protein detects NANBV-specific antibodies as early as 14 weeks post inoculation, whereas the C100-3-based immunoassay does not detect any anti-NANBV antibody at the times studied.

In Table 4, results are presented from ALT, EM, anti-HCV, and anti-CAP-N assays on sera from a chimpanzee with a self limited infection presented.

TABLE 4

CHIMP 213 - SELF LIMITED INFECTION

Week Post Inoc	ALT	EM	Anti HCV	Anti CAP-N
4	24	+	-	+
6	34	+	-	+
8	38	+	-	+
13	28	ND	-	+
16	25	ND	-	+
18	23	ND	+	+
20	25	-	+	+

The results in Table 4 show that the CAP-N antigen detects anti-NANBV antibodies earlier than the C100-3 antigen when using sera sampled during the course of a self-limiting NANBV infection.

In Table 5, results are presented from ALT, anti-HCV and anti-CAP-N assays on sera from a chimpanzee that converted from an acute infection profile to a chronic one.

TABLE 5

CHIMP 10 - ACUTE/CHRONIC NANB HEPATITIS

Symptoms	Week Post Inoc	Peak ALT	Anti HCV	Anti CAP-N
acute	2	223	-	+
chronic	40	223	+	+
chronic	42	223	+	+
chronic	44	223	+	+
chronic	51	223	+	-

The results in Table 5 indicate that the CAP-N antigen preferentially detects anti-NANBV antibodies in acute stages of NANBV infection.

In Table 6, results are presented from ALT, EM, anti-HCV (anti-C100-3) and anti-CAP-N assays on sera collected at various intervals from several chimpanzees with acute or chronic NANB Hepatitis.

TABLE 6

Week Post Inoc	Week Post Alt Elev	Peak ALT	Anti HCV	Anti CAP-N
ADDITIONAL ACUTE SERA				
2	+1	73	-	+
14	+2	66	-	+
6	+2	197	-	+
11	+1	151	-	-
8	+4	125	-	+
15	+1	82	-	+
12	-4	73	ND	+
ADDITIONAL CHRONIC SERA				
156	+131	110	+	+
156	-	89	+	+
160	-	89	+	+

The results in Table 6 indicate that the CAP-N antigen more often detected anti-NANBV antibodies in sera from acutely infected individuals than did the C100-3 antigen.

The results of Tables 2-6 show that the NANBV structural protein of the invention, in the form of a fusion protein containing CAP-N antigen and produced by the vector pGEX-3X-690:694, detects antibodies in

defined seraconversion series at times in an infected patient or chimpanzee earlier than detectable by present state of the art methods using the C100-3 antigen. In addition, the results show that CAP-N antigen is particularly useful to detect acute NANBV infection early in the infection.

Taken together, the results indicate that patients infected with NANBV contain circulating antibodies in their blood that are immunospecific for NANBV antigen designated herein as structural antigens, and particularly are shown to immunoreact with the putative capsid antigen defined by CAP-N. These antibodies are therefore referred to as anti-NANBV structural protein antibodies and are to be distinguished from the class of antibodies previously detected using the NANBV non-structural protein antigen C100-3.

In Table 7, comparative results are presented from anti-HCV capsid fusion protein assays according to the basic immunoblot assay described in Example 4 using various chimp and human sera on the following HCV capsid fusion proteins: CAP-N, CAP-A, CAP-B and CAP-C.

TABLE 7

SERA	TYPE[a]	CAP-N[b]	CAP-A[c]	CAP-B[d]	CAP-C[e]
C18	Chimp 10 (A)	+++	+	+	-
C10	Chimp 194(A)	+++	+++	+++	-
59-16	Chimp 59 (A)	+++	+	+++	ND
59-12	Chimp 59 (A)	ND[f]	++	+++	-
C9	Chimp 181(A)	+++	-	+++	-
213-18	Chimp 213(A)	ND	+	+	-
C2	Chimp 10 (C)	++	-	-	-
C1	Chimp 10 (C)	+++	-	-	-
C19	Chimp 10 (C)	+++	-	-	-
C4	Chimp 68 (C)	+++	+++	+++	ND
169-16	Human	ND	+++	+++	-
169-23	Human	ND	+++	+++	-
191-1	Human	+	+	+	ND
191-2	Human	+	+	++	ND
191-3	Human	+	+	+	ND
216-1	Human	-	+/-	+/-	ND
216-2	Human	+	+	+	ND
216-3	Human	+	+	+	ND

a The type of sera tested is indicated by the species (chimp or human), a chimp identification number if the sample is from a chimp, and a designation (in parenthesis) if the sera donor exhibits acute (A) or chronic (C) HCV infection at the time the sera was sampled.

b CAP-N indicates the GST:NANBV 690:694 fusion protein produced in Example 5 that includes HCV capsid protein residues 1 to 74.

c CAP-A indicates the GST:NANBV fusion protein produced in Example 5 that includes HCV capsid protein residues 1 to 20.

d CAP-B indicates the GST:NANBV fusion protein produced in Example 5 that includes HCV capsid protein residues 21 to 40.

e CAP-C indicates the GST:NANBV fusion protein produced in Example 5 that includes HCV capsid protein residues 41 to 60.

f +, ++ and +++ indicate relative amounts of anti-HCV capsid antibody immunization product detected by the Western blot assay, where + indicates a weak band after overnight exposure of the x-ray film, ++ indicates a strong band after overnight exposure of the x-ray film, +++ indicates a strong band after 1 to 2 hours exposure of the X-ray film, and +/- or - indicates a faint or no band, respectively, after overnight exposure of the X-ray film

g "ND" indicates not tested.

The results shown in Table 7 indicate that fusion proteins containing the CAP-A antigen or CAP-B antigen are immunoreactive with antibodies present in sera from HCV-infected humans or chimps. In addition, CAP-C antigen does not significantly immunoreact with sera from HCV infected humans or chimps.

Example 8. Characterization of NANBV Genomic RNA Sequence

A. Characterization of cDNA Clones and Primary Structure of NANBV

(1) Isolation of NANBV Viral RNA.

NANBV, also referred to as hepatitis C virus (HCV), was isolated from two tissue sources from a HCV-infected chimpanzee, number 59 (c59), that had been inoculated with the Hutch (H) strain of HCV (designated HCV-Hc59) as described in Example 1A(1). Chimpanzee liver was biopsied during the acute phase

of infection (4 weeks post-inoculation) and chimpanzee plasma was taken 13 weeks post-inoculation. Extraction of nucleic acids from liver was performed as described by Ogata et al., Proc. Natl. Acad. Sci., USA, 88:3392-3396 (1991) and in Example 1A(6). HC virions were isolated from plasma having viral titers of $10^{5.5}$ to $10^{6.5}$ CID_{50}/ml. HCV RNA was purified from the plasma samples by either immunoaffinity chromatography as described in Example 1A(1) or by isopropanol precipitation.

Briefly, 50 µl of plasma was diluted with an ice cold buffer solution containing 4.2 M guanidinium isothiocyanate, 0.5% sarcosyl and 0.025 M Tris-HCl at pH 8.0. The diluted plasma was then admixed with 50 µl of extraction buffer containing 100 mM Tris-HCl at pH 8.0, 10 mM EDTA and 1% SDS to form an extraction admixture. The admixture was vortexed and maintained at 5 minutes at 65°C to initiate extraction. Serum proteins were then removed from the admixture with phenol/chloroform at 65°C followed by one extraction with chloroform alone. HCV RNA was then precipitated from the protein-free admixture by admixing two volumes of ice cold isopropanol and one-tenth volume of 3 M sodium acetate and maintaining the admixture overnight at -20°C. After pelleting by centrifugation in an Eppendorf centrifuge at 1400 rpm for 30 minutes at 4°C, HCV RNA was washed once with 70% ethanol, vacuum dried and then resuspended in 9 µl RNAse-free water. Purified HCV RNA samples were heated for 5 minutes at 65°C prior to cDNA synthesis performed as described below and in Example 1A(1).

(2) Cloning of HCV-Hc59 cDNA.

Five µg of purified liver or plasma derived HCV RNA was used per cDNA priming reaction. Specific nucleotide primers derived from published HCV

sequences and spanning the entire reported genomic sequences were used to prime the reaction. See Okamoto et al., Japan. J. Exp. Med., 60:167-177 (1990); Kato et al., Proc. Natl. Acad. Sci., USA, 87:9524-9528 (1990); Han et al., Proc. Natl. Acad. Sci., USA, 88:1711-1715 (1991); and Houghton et al., European Patent Application Number 88310922.5 and Publication Number 318216. Selected target sequences were amplified using a PCR-based approach using a variety of nucleotide primers as described in Example 1A(3). The nucleotide sequences of the primers are listed in Table 8 below and have been identified by primer number and corresponding SEQ ID NOs.

TABLE 8

NUCLEOTIDE PRIMERS USED IN CLONING HCV-HC59 CDNA

PRIMER (#)	SEQ ID NO.	NUCLEOTIDE SEQUENCE (5'-3')	POLARITY[a]
1	47	CAGCCCCCTGATGGGGGCGAC	+
22	48	ACTCGCAAGCACCCTATCA	-
21	49	CTGTGAGGAACTACTGTCT	+
690	50	ATGAGCACGAATCCTCAAACCT	+
694	51	GTCCTGCCCTCGGGCC	-
693	52	CGAGGAAGACTTCCGAGC	+
691	53	ACCCAAATTGCGCGACCTAC	-
15	54	TAAGGTCATCGATACCCT	+
17	55	CAGTTCATCATCATATCCCA	-
18	56	AGATAGAGAAAGAGCAAC	-
23	57	AGACTTCCGAGCGGTCGCAA	+
717	58	GACCTGTGCGGGTCTGTC	+
567	59	GGGTCGGCAGCTGGCTAGCCTCTCA	-
801	60	TCCTGGCGGGCATAGCGT	+
8	61	CCCCAGCCCTGGTCAAAATCGGTAA	-
568	62	TGAGAGGCTAGCCAGCTGCCGACCC	+

	745	63	CTGTCGGTCGTTCCCACCA	-
	626	64	CCGCGAAGAGTGTGTGTGGT	+
	627	65	CAATGTTCTGGTGGAGGTG	-
	617	66	GCCATTAAGTGGGAGTACGTCGTTCTCC	+
5	652	67	CGAGGAAGGATACAAGACC	-
	628	68	TGCTTGTGGATGATGCTACT	+
	629	69	CACACGTGCAGTTGCGCT	-
	701	70	CTGCTGACCACTACACAG	+
	654	71	GACCAGAGTGGAAGCGCAA	+
10	653	72	TACCAGAGTCGGGTGTACAG	-
	500	73	CTAGGAGGCCCCTTGTCTGC	-
	688	74	CTCGGGCCAGCCGATGGA	+
	633	75	GGGGACCTCATGGTTGTCT	-
	846	76	CCCGTGGAGTGGCTAAGG	+
15	831	77	CTCCTCGATGTTGGGATGG	-
	830	78	CAGAGCTTCCAGGTGGCTC	+
	795	79	CGGGCTCCGTCACTGTG	+
	794	80	GTATTGCAGTCTATCACCGAG	-
	464	81	GGCTATACCGGCGACTTCGA	+
20	40	82	CGTTGAGTGCGGGAGACAG	-
	463	83	TCACCATTGAGACAATCACG	+
	788	84	GTAAGGAAGGTTCTCCCCACTC	-
	571	85	ATGCCCACTTTCTATCCCAGACAAAGC	+
	623	86	TGCATGTCATGATGTAT	-
25	841	87	GGACAAGACGACCCTGCC	-
	625	88	CGTATTGCCTGTCAACAGGC	+
	631	89	AGCGCCCACAAAGGCAGTAG	-
	842	90	CCTCTTCAACATATTGGGG	+
	843	91	CCAGGAACCGGAGCATGG	-
30	859	92	ACCAGTGGATAAGCTCGG	+
	904	93	CGTGGTGTAGGCATTAATG	-
	862	94	ATGTGGAGTGGGACCTTCC	+
	861	95	CTCTGCTGTTATATGGGAGG	-
	F4	96	GTTGACGTCCATGCTCACTG	+
35	A4	97	TTTCCACGTCTCCACTAGCG	-

849	98	GTGAGGACCACCGTCCGC	-
F1	99	TTCCACCTCCAAAGTCCCCT	+
2_1	100	AGAACTTGCAGTCTGTCAAATGTGA	-
621	101	GGAAGAACAGAAACTGCCCATCAATGCACTAAGC	+
2_0	102	TGACGCCGCTGCTTTAACCT	-
2_2	103	TGCAAGCTTCCTCTACGGAT	-
51	104	AGGTTAAAGCAGCGGCGTCA	+
50	105	AGCTTCCCATCACGGCCAA	-
502	106	GATGGCTTTGTACGACGTG	+
55	107	GCACCTGCGATAGCCGCAGT	-
852	108	GTCCCTCACCGAGAGGCT	+
853	109	GATTGGAGGTAGATCAAGTG	-
4	110	TACGACTTGGAGCTCATAAC	+
62	111	AGCAAGACACACTCCAGTCA	+
61	112	GCCTATTGGCCTGGAGTGGTTAGC	-

- (+) indicates sense strand
  (-) indicates anti-sense strand

Amplified sequences were subsequently isolated, rendered blunt-ended and inserted into a pUC or pBluescript (Stratagene) cloning vectors by standard procedures as described in Example 1A(4).

(3) Sequence Analysis of Cloned HCV-Hc59 cDNA

Clones were sequenced using the dideoxy chain termination method using a duPont automated sequencer Genesis 2000. In order to minimize sequencing errors due to PCR artifacts (misreading by Taq polymerase), three independent clones were isolated for each target sequence and were then sequenced. The resulting sequences were compared in order to derive the final consensus sequence representative of the HCV Hutch strain (HCV-H) genome. In some cases, several clones derived from independent studies encompassed the same

genomic domain. The sequences of these clones provided further confirmatory data.

(4) Characterization of cDNA Clones and Primary Structure of HCV-Hc59

Pairs of primers were selected as described above and in Example 1A(3) to amplify specific regions of the HCV-Hc59 genome to generate overlapping clones, the sequences of which would comprise the entire genome. The primer pairs used in specific PCR reactions are listed in Table 9 below. The resultant forty cDNA clones generated from the selected primer pairs are listed numerically beginning with zero and ending with 39 in the same table and correspond to the putative map location shown in Figure 1. The deduced size in base pairs of each isolated cDNA is also listed in Table 9.

TABLE 9

PCR DERIVED HCV-HC59 CLONES

Clone #[a]	Primer Pair[b]	Insert Size (bp)[c]
0	1:22	309
1	21:22	268
2	690:694	224
3	693:691	216
4	15:18	170
5	23:18	378
6	15:17	618
7	717:567	548
8	801:8	346
9	568:745	205
10	626:627	597
11	617:652	173
12	628:652	119
13	628:629	390
14	701:652	314

15	654:653	106
16	654:500	572
17	688:633	590
18	846:831	537
19	830:831	432
20	795:794	313
21	464:40	134
22	463:788	347
23	571:623	241
24	571:841	362
25	625:631	482
26	842:843	568
27	859:904	320
28	862:861	390
29	F4:A4	397
30	F4:849	498
31	F1:2_1	493
32	621:2_1	132
33	621:2_0	181
34	621:2_2	221
35	51:50	360
36	502:55	322
37	852:853	625
38	4:853	315
39	62:61	611

[a] Relative location on HCV-Hc59 genome shown in Figure 1.

[b] Sense (+) and anti-sense (-) primer pairs having nucleotide sequences shown in Table 1 and in the Sequence Listings.

[c] Deduced size in base pairs (bp) of the cloned insert produced by PCR using the indicated primer pair as described in Example 1A(3) and 8A(3).

Comparison of the sequences of three independently isolated cDNA clones from the same genomic domain revealed very few nucleotide differences indicating that the virus stock was homogeneous. The sequence of the complete HCV-H genome was deduced, representing 9416 nucleotides, which is similar in length to that of previously isolated HCV genomes, HCV-1, HCV-J, and HCV-BK. See, Kato et al., supra; Choo et al., Proc. Natl. Acad. Sci., USA, 88:2451-2455 (1991); and Takamizawa et al., J. Virol., 65:1105-1113 (1991). The sequence has a high GC content (58.8%), and contains one large open reading frame beginning at nucleotide base number 342 and ending at nucleotide base number 9374 (SEQ ID NO:46) corresponding to a protein of 3011 amino acid residues (SEQ ID NO:46). The deduced nucleotide sequences of HCV-Hc59 have been deposited in GenBank having the accession number M67463.

HCV-Hc59 sequences from the 5' and 3' end terminal non-coding (NC) domains, respectively encompassing 341 and 42 nucleotides, were identified. The first 12 nucleotides and the last 20 nucleotides (SEQ ID NO:46-see features) correspond to the nucleotide primers used in the amplification process and, thus are not confirmed as HCV-H sequences. However, 5' non-coding sequences of previously reported HCV genomes are extremely conserved (>98%), making it likely that the 5' end sequence of HCV-H reported here is very similar if not identical to the one indicated. However, due to greater divergence among HCV- 3' non-coding sequences, the HCV-Hc59 3' end sequence remains subject to confirmation. When an oligo (dT) primer was used for cDNA synthesis followed by PCR amplification using different combinations of primers, no viral sequences were obtained. This

result indicates that the viral genome lacks internal A-rich tracts at the 3' terminal end or a 3'-terminal poly (rA) sequence. Similarly, no sequences were amplified when A-rich primers complementary to the 3' end (U-rich) nucleotide sequence of the two reported Japanese isolates, HCV-J and HCV-BK, were used in the RT priming reaction, thus suggesting the absence of a U-rich terminal sequence in the genome of HCV-Hc59.

The large open reading frame of the HCV-Hc59 RNA genome is preceded by five AUG codons (cDNA = ATG - nucleotide base numbers 13, 32, 85, 96 and 214 as shown in SEQ ID NO:46) confirming the existence of hypothetical small open reading frames in the 5' NC region of HCV genomes. Several repeated sequences as shown in SEQ ID NO:46 listed as R_1 through R_5 in the features portion of the listing were identified in the 5' and the 3' NC regions, and in the C terminal of the putative NS5 domain. These sequences might correspond to important Cis acting elements involved in the regulation of viral replication.

The repeated sequences, R_2 and R_3, appear conserved among all HCV isolates. Although other repeated sequences have now been found in the terminal ends of HCV genomes, it is possible that sequences having a regulatory function would be sequences conserved among all HCV viruses, such as R_2 and R_3. The repeated sequence R_2 is particularly significant as it is represented by the highest copy number of four, is found within both the 5' and 3' terminal ends, and is localized upstream from a 3' terminal hairpin loop which may be involved in cyclization of viruses. Nothing is yet known about putative cyclization of HCV viruses. It is also possible that these very conserved self-complementary sequences may represent replicase recognition sites, possibly used

for both the plus and minus strands of the viral genome.

As described in previous reports for other HCV isolates, (Kato et al., Proc. Natl. Acad. Sci. USA, 87:9524-9528 (1990); Choo et al., Proc. Natl. Acad. Sci. USA, 88:2451-2455 (1991); and Takamizawa et al., J. Virol., 65:1105-1113 (1991)) the HCV-Hc59 genome or protein shares only limited similarity with other known viral sequences, except for three domains: (1) a few stretches of nucleotides in the 5' NC sequence are conserved with pestiviruses identical to those reported by Choo et al., supra, for the American prototype HCV-1 (SEQ ID NO:46), (2) blocks of amino acids found in the putative NS3 domain (nucleotide base numbers 3693 to 5198; SEQ ID NO:46) corresponding to putative NTP-binding helicase and trypsin-like serine proteases are conserved with flaviviruses and pestiviruses; and (3) the GDD consensus sequence conserved among all viral-encoded RNA-dependent RNA polymerases (amino acid residues 2737 to 2739; SEQ ID NO:46). In addition, a total of nineteen putative N-glycosylation sites were located, essentially clustered between amino acid residues 196 and 647 in a similar fashion to the organization observed for the envelope proteins of pestiviruses as described by Meyers et al., Virol., 171:555-567 (1989); and Collett et al., Virol., 162:167-180 (1988).

B. Comparison of Nucleotide and Protein Sequences of HCV-Hc59 and Heterologous HCV Isolates

A summary of the comparison between different genomic domains of HCV-Hc59 and the previously reported sequences for the American (HCV-1) or American-like (HC-J1) isolates, and for the Japanese isolates HC-J4, HCV-JH, HCV-J and HCV-BK is shown in

Table 10. Sequence comparison is limited with HC-J1, HC-J4 and HC-JH as the complete sequence of the genome of these isolates has not yet been reported. The hypothetical map assignments for HCV-encoded proteins deduced from sequence and hydrophobicity profile similarity between HCV genomes and flaviviruses and/or pestiviruses were used for making the comparison. The references for the compared sequences are listed at the bottom of Table 10. Based on sequence comparisons to related viruses, the HCV genome is believed to encode at least 8 domains as indicated in Table 10 : the structural domain consisting of the nucleocapsid (C) and two envelope (E1 and E2) proteins, and the non-structural region consisting of five proteins, NS2, NS3, NS4a, NS4b, and NS5. Domain designations are based on the organization of related HCV strains for comparative purposes, and do not necessarily reflect the domains of HCV-Hc59 because of the present state of the art in characterizing the domains of HCV-Hc59.

TABLE 10

HOMOLOGY OF NUCLEOTIDE AND DEDUCED AMINO ACID SEQUENCE BETWEEN HCV-Hc59 AND HETEROLOGOUS ISOLATES

Domain[1]	Isolate[2]					
	HCV-1	HC-J1	HC-J4	HCV-JH	HCV-J	HCV-BK
5'NC -326-1 % bp[3]	99.7	99.1	99.1	98.9	98.2	98.8
C 1-570 bp	98.4	98.9	90.0	90.3	91.0	90.3
C 1-570 aa	98.9	98.9	97.9	98.4	98.9	98.4
E1 571-1140 bp	93.5	93.1	74.1	73.7	73.9	73.8
E1 571-1140 aa	94.1	93.2	78.9	79.4	78.8	77.9
E2/NS1 1141-2197 bp	93.6	91.7	67.7	65.4	73.5	71.2
E2/NS1 1141-2197 aa	92.9	88.7	70.7	65.6	79.3	80.4
NS2 2198-3350 bp	93.8	--	--	--	72.4	72.7
NS2 2198-3350 aa	95.1	--	--	--	80.0	78.2
NS3 3351-4856 bp	95.4	--	--	--	80.1	78.9
NS3 3351-4856 aa	97.2	--	--	--	92.2	92.6

	HCV-1	HC-J1	HC-J4	HCV-JH	HCV-J	HCV-BK
NS4a 4857-5596						
bp	95.8	--	--	--	80.4	80.0
aa	95.5	--	--	--	87.0	86.2
NS4b 5597-6049						
bp	95.4	--	--	--	76.9	77.7
aa	96.7	--	--	--	84.8	85.4
NS5 6050-9036						
bp	95.9	--	--	--	78.3	79.3
aa	96.7	--	--	--	83.2	83.7
3'NC 9037-9055						
bp	83.3	--	--	--	73.6	63.1

[1] Nucleotide position for C and E1 deduced from Weiner et al., Virol., 180:842-848 (1991) and for E_2 and NS2-NS5 from Takamizawa et al., J. Virol., 65:1105-1113 (1991);

[2] The nucleotide positions are calculated from the AUG initiation codon where A is base number 1.

[3] The percentage of homology in base pairs (bp) and amino acid (aa) is listed.

The data indicate a very high degree of identity found in two genomic domains (5' NC and C) for all isolates despite geographical separation (90.0-98.9% nucleotide homology and 97.9 to 98.9% amino acid homology). A similar observation has been made in

flaviviruses that are members of the same sero-related subgroup by Brinton et al., Virol., 162:290-299 (1988), whereas members of different antigenic subgroups share only low levels of homology in that region. Two sets of repeated sequences found in the 5' NC domain, R_2 and R_3 (SEQ ID NO:46), are conserved among all reported isolates. Two copies of the repeated sequence R_1 are also conserved between the two American isolates HCV-Hc59 and HCV-1 but only one copy is found in both Japanese isolates HCV-J and HCV-BK. The 5' NC sequence of these genomes does not extend far enough to encompass the second copy. The nucleotide sequence reported for the other HCV isolates does not extend far enough into the 5' NC to allow for comparison.

Regions of moderate identity were found throughout the non-structural domains, where a clear separation between the two groups (American/Japanese) isolates could be seen. Whereas 93.8 to 95.9% nucleotide identity was observed when HCV-Hc59 was compared with the first group, only 72.7 to 80.0% identity was found with the second group (95.1 to 97.2% and 78.2-92.6% amino acid identity, respectively). One region, found in the putative NS5 (amino acid residue position 2356 to 2379 of SEQ ID NO:46) and called Region V_3 as shown in Table 11 below reflected even a more striking divergence between the two subgroups of HCV isolates. This region showed 100% identity between the two American isolates (data not shown) but only 12.5% with either Japanese strains. Although most of the changes appear to be conservative changes and might not therefore result in functional modification of the protein, it would be of interest to assess whether this genomic region is

immunologically active and if antigenic variation also exist between the two subgroups of HCV isolates.

Table 11[1]

REGION V

(Residues 386 to 411 of SEQ ID NO:46)

Isolates[2]

HCV-Hc59:

His Val Thr Gly Gly Asn Ala Gly Arg Thr Thr Ala Gly Leu Val Gly Leu Leu Thr Pro Gly Ala Lys Gln Asn Ile (SEQ ID NO:113)

HCV-1:

His Val Thr Gly Gly Ser Ala Gly His Thr Val Ser Gly Phe Val Ser Leu Leu Ala Pro Gly Ala Lys Gln Asn Val (SEQ ID NO:114)

HC-J1:

His Val Thr Gly Gly Gln Ala Ala Arg Ala Met Ser Gly Leu Val Ser Leu Phe Thr Pro Gly Ala Lys Gln Asn Ile (SEQ ID NO:115)

HCV-J:

His Val Thr Gly Gly Arg Val Ala Ser Ser Thr Gln Ser Leu Val Ser Trp Leu Ser Gln Gly Pro Ser Gln Lys Ile (SEQ ID NO:116)

HCV-BK:

His Val Thr Gly Gly Ala Gln Ala Lys Thr Thr Asn Arg Leu Val Ser Met Phe Ala Ser Gly Pro Ser Gln Lys Ile (SEQ ID NO:117)

HC-J4:

Tyr Thr Ser Gly Gly Ala Ala Ser His Thr Thr Ser Thr Leu Ala Ser Leu Phe Ser Pro Gly Ala Ser Arg Asn Ile (SEQ ID NO:118)

HCV-JH:

His Val Thr Gly Gly Val Gln Gly His Val Thr Ser Thr Leu Thr Ser Leu Phe Arg Pro Gly Ala Ser Gln Lys Ile (SEQ ID NO:119)

HCV-Hh-H77:

His Val Thr Gly Gly Ser Ala Gly Arg Thr Thr Ala Gly Leu Val Gly Leu Leu Thr Pro Gly Ala Lys Gln Asn Ile (SEQ ID NO:120)

HCV-Hh-H90:

His Val Thr Gly Gly Ser Ala Gly Arg Ser Val Leu Gly Ile Ala Ser Phe Leu Thr Arg Gly Pro Lys Gln Asn Ile (SEQ ID NO:121)

REGION V_1
(Residue 246 to 275 of
SEQ ID NO:46)

HCV-Hc59:

Val Ala Thr Arg Asp Gly Lys Leu Pro Thr Thr Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu (SEQ ID NO:122)

HCV-1:

Val Ala Thr Arg Asp Gly Lys Leu Pro Ala Thr Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu (SEQ ID NO:123)

HC-J1:

Val Ala Thr Arg Asp Gly Lys Leu Pro Ala Thr Gln
Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala
Thr Leu Cys Ser Ala Leu (SEQ ID NO:123)

HCV-J:

Leu Ala Ala Arg Asn Ser Ser Ile Pro Thr Thr Thr
Ile Arg Arg His Val Asp Leu Leu Val Gly Ala Ala
Ala Leu Cys Ser Ala Met (SEQ ID NO:124)

HCV-BK:

Leu Ala Ala Arg Asn Val Thr Ile Pro Thr Thr Thr
Ile Arg Arg His Val Asp Leu Leu Val Gly Ala Ala
Ala Phe Cys Ser Ala Met (SEQ ID NO:125)

HC-J4:

Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr
Ile Arg Arg His Val Asp Leu Leu Val Gly Ala Ala
Ala Phe Cys Ser Ala Met (SEQ ID NO:126)

HCV-JH:

Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr
Leu Arg Arg His Val Asp Leu Leu Val Gly Thr Ala
Ala Phe Cys Ser Ala Met (SEQ ID NO:127)

HCV-Hh-H77:

Val Ala Thr Arg Asp Gly Lys Leu Pro Thr Thr Gln
Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala
Thr Leu Cys Ser Ala Leu (SEQ ID NO:122)

HCV-Hh-H90:

Val Ala Thr Arg Asp Gly Lys Leu Pro Thr Thr Gln
Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala
Thr Leu Cys Ser Ala Leu (SEQ ID NO:122)

REGION V_2
(Residue 456 to 482 of
SEQ ID NO:46)

HCV-Hc59:
Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Ala Gln
Gly Trp Gly Pro Ile Ser Tyr Ala Asn Gly Ser Gly
Leu Asp Glu (SEQ ID NO:128)

HCV-1:
Leu Ala Ser Cys Arg Pro Leu Thr Asp Phe Asp Gln
Gly Trp Gly Pro Ile Ser Tyr Ala Asn Gly Ser Gly
Pro Asp Gln (SEQ ID NO:129)

HC-J1:
Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Asp Gln
Gly Trp Gly Pro Ile Ser His Ala Asn Gly Ser Gly
Pro Asp Gln (SEQ ID NO:130)

HCV-J:
Met Ala Ser Cys Arg Pro Ile Asp Glu Phe Ala Gln
Gly Trp Gly Pro Ile Thr His Asp Met Pro Glu Ser
Ser Asp Gln (SEQ ID NO:131)

HCV-BK:
Met Ala Gln Cys Arg Thr Ile Asp Lys Phe Asp Gln
Gly Trp Gly Pro Ile Thr Tyr Ala Glu Ser Ser Arg
Ser Asp Gln (SEQ ID NO:132)

HC-J4:
Met Ala Ser Cys Arg Pro Ile Gln Trp Phe Ala Gln
Gly Trp Gly Pro Ile Thr Tyr Thr Glu Pro Asp Ser
Pro Asp Gln (SEQ ID NO:133)

HCV-Hh-H77:

Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Ala Gln
Gly Trp Gly Pro Ile Ser Tyr Ala Asn Gly Ser Gly
Leu Asp Glu (SEQ ID NO:128)

HCV-Hh-H90:

Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Asp Gln
Gly Trp Gly Pro Ile Ser Tyr Ala Asn Gly Ser Gly
Pro Asp Glu (SEQ ID NO:134)

REGION V_3
(Residue 2356 to 2379 of
SEQ ID NO:46)

HCV-Hc59:

Ser Thr Ser Gly Ile Thr Gly Asp Asn Thr Thr Thr
Ser Ser Glu Pro Ala Pro Ser Gly Cys Pro Pro Asp
(SEQ ID NO:135)

HCV-J:

Gly Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Gly
Pro Pro Asp Gln Ala Ser Asp Asp Gly Asp Lys Gly
(SEQ ID NO:136)

HCV-BK:

Glu Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Ala
Leu Pro Asp Gln Ala Ser Asp Asp Gly Asp Lys Gly
(SEQ ID NO:137)

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1 Alignment of the deduced amino acid residue sequence of Regions V, V_1, V_2, and V_3 of HCV-Hc59 with other American and Japanese isolates.

2 Isolates:
HCV-Hc59: American/Chimp 59; Inschauspe et al., Proc. Natl. Acad. Sci., USA, 1991; GenBank Accession Number M67463;

HCV-1: American; Choo et al., Proc. Natl. Acad. Sci., USA, 88:2451-2455 (1991); GenBank Accession Number M62321;

HCV-1: 5' termini - Han et al., Proc. Natl. Acad. Sci. USA, 88:1711-1715 (1991); Genbank Accession Number M58407;

HCV-1: 3' termini - Han et al., supra; GenBank Accession Number M58406;

HC-J1: American; Okamoto et al., Japan J. Exp. Med., 60:167-177 (1990);

HCV-J: Japanese; Kato et al., Proc. Natl. Acad. Sci., USA, 87:9524-9528 (1990); Genbank Accession Number D90208;

HCV-BK: Japanese; Takamizawa et al., J. Virol., 65:1105-1113 (1991); Genbank Accession Number M58335;

HC-J4: Japanese; Okamoto et al., supra;

HCV-JH: Japanese; Takeuchi et al., Nucl. Acids Res., 18:4626 (1990);

HCV-Hh-H77 and H90: American/human; Ogata et al., Proc. Natl. Acad. Sci., USA, 88:3392-3396 (1991).

Regions of greater divergence were found in the putative envelope E1 (nucleotide base number 571 to 1140) and E2 (nucleotide base number 1141 to 2197 as calculated from the AUG initiation codon), where 77.9 to 94.1% and 65.6 to 92.9% amino acid identity, respectively, was observed between HCV-Hc59 and the other isolates. In addition to the moderate and hypervariable regions identified by Weiner et al., Virol., 180:842-848 (1991) in E1 and E2 (amino acid residues 214 to 254 and 386 to 411, respectively) for which protein heterogeneity between HCV-Hc59 and other

HCV isolates ranged from 70.7 to 97.6% for the moderate region (data not shown) and from 51.7 to 72.4% for Region V as shown in Table 11, two regions of high variability were identified. Both regions, Region V_1 and Region V_2 (amino acid residues 246 to 275 and 456-482, respectively) appeared very conserved among American or Japanese type HCV (96% identity) but showed striking heterogeneity when both groups were compared (55-58% protein identity, Table 11). In contrast to the observation made by Weiner et al., supra, who reported that approximately 50% of the amino acid changes observed in Region V between four American isolates and one Italian isolate are non-conservative changes, more than 85% of the changes observed in either Region V, V_1 or V_2 were found to consist of conservative changes. Although the function of these regions remain unknown, these data suggest that they are under immunological pressure and could be good candidates for targeting protective epitope domains that might be subtype specific in the case of Regions V_1 and V_2.

Thus, the genome of HCV-Hc59 shows an overall amino acid homology of 96% with the American prototype HCV-1 and 84.9% with both HCV-J and HCV-BK isolates. Three new regions of high variability were identified within E1, E2 and NS5 (Regions V_1, V_2 and V_3, respectively). In all three regions, sequence heterogeneity appears to be subgroup specific (i.e., American versus Japanese isolates), in particular for Region V_3 where up to 87.5% divergence was found between the two subgroups as shown in Tables 10 and 11. Sequence heterogeneity has been observed in the envelope/NS1 regions of flaviviruses (see, Meyers et al., Virol., 171:555-567 (1989); Collett et al., Virol., 165:191-199 (1988); and Hahn et al., Virol.,

162:167-180 (1988) but not to the extent reported here for Regions V_1 and V_2, thus further suggesting that HCV structure is significantly divergent from this family of viruses. The fact that three of four variable regions of the HCV genome are located in the putative envelope domains confirm that these domains are under great immunological pressure possibly associated with evolutionary-linked molecular divergence. A high rate of nucleotide change (28.2%) in the putative E2/NS1 domain of HCV-H over an interval of thirteen years suggests significant evolution of the HCV genome in that domain. See Ogata et al., supra.

The cDNA sequence of the human prototype strain H of HCV (9416 nucleotides) is the subject of this invention. To date, this is the second nucleotide sequence of a HCV genome determined for a prototype strain, as the two reported Japanese sequences HCV-J and HCV-BK have been derived from clones isolated from a mixture of plasma therefore representing likely genomic sequences from multiple isolates. The data confirms that HCV exhibits a unique structure and organization more closely related to the pestiviruses than flaviviruses by the presence of stretches of nucleotides highly conserved in the 5' NC domain, putative small open reading frames preceding the initial AUG codon, and putative NTP-binding helicases or trypsin-like serine proteases.

Description of SEQ ID NO:1-6 in the Sequence Listings

SEQ ID NO:1 contains the linear single-stranded nucleotide base sequence of a preferred DNA segment of the present invention that encodes portions of the structural proteins of the Hutch strain of NANBV. The base sequences are shown conventionally from left to

right and in the direction of 5' terminus to 3' terminus using the single letter nucleotide base code (A=adenine, T=thymine, C=cytosine and G=guanine) with the position number of the first base residue in each row indicated to the left of the row showing the nucleotide base sequence.

The reading frame of the nucleotide sequence of SEQ ID NO:1 is indicated by placement of the deduced amino acid residue sequence of the protein for which it codes below the nucleotide sequence such that the triple letter code for each amino acid residue (Table of Correspondence) is located directly below the three bases (codon) coding for each residue. SEQ ID NO:1 also contains the linear amino acid residue sequence encoded by the nucleotide sequence of SEQ ID NO:1 and is shown conventionally from left to right and in the direction of amino terminus to carboxy terminus. The position number for every fifth amino acid residue is indicated below that amino acid residue sequence.

SEQ ID NO:2 contains the linear amino acid residue sequence of a preferred fusion protein designated CAP-N and is comprised of an amino-terminal polypeptide portion corresponding to residues 1 to 221 of glutathione-S-transferase, an intermediate polypeptide portion corresponding to residues 222 to 225 and defining a cleavage site for the protease Factor Xa, a linker portion corresponding to residues 226 to 234, a polypeptide portion corresponding to residues 235 to 308 defining a NANBV capsid antigen that has the amino acid residue sequence 1 to 74 in SEQ ID NO:1, and a carboxy-terminal linker portion corresponding to residues 309 to 315. SEQ ID NO:2 also contains the nucleotide base sequence of a linear single-stranded DNA segment that encodes the fusion protein described herein. The nomenclature and

presentation of sequence information is as described for SEQ ID NO:1.

SEQ ID NO:3 contains the linear amino acid residue sequence of a preferred fusion protein designated CAP-A and comprised of an amino-terminal polypeptide portion corresponding to residues 1 to 220 of glutathione-S-transferase, an intermediate polypeptide portion corresponding to residues 221 to 226 and defining a cleavage site for the protease Thrombin, a polypeptide portion corresponding to residues 227 to 246 defining a portion of the NANBV capsid antigen that has the amino acid residue sequence 1 to 20 in SEQ ID NO:1, and a carboxy-terminal linker portion corresponding to residues 247 to 252. SEQ ID NO:3 also contains the nucleotide base sequence of a linear single-stranded DNA segment that encodes the fusion protein described therein. The nomenclature and presentation of sequence information is as described for SEQ ID NO:1.

SEQ ID NO:4 contains the linear amino acid residue sequence of a preferred fusion protein designated CAP-B and comprised of an amino-terminal polypeptide portion corresponding to residues 1 to 220 of glutathione-S-transferase, an intermediate polypeptide portion corresponding to residues 221 to 226 and defining a cleavage site for the protease Thrombin, a polypeptide portion corresponding to residues 227 to 246 defining a portion of the NANBV capsid antigen that has the amino acid residue sequence 21 to 40 in SEQ ID NO:1, and a carboxy-terminal linker portion corresponding to residues 247 to 252. SEQ ID NO:4 also contains the nucleotide base sequence of a linear single-stranded DNA segment that encodes the fusion protein described therein. The

nomenclature and presentation of sequence information is as described for SEQ ID NO:1.

SEQ ID NO:5 contains the linear amino acid residue sequence of a preferred fusion protein designated CAP-C and comprised of an amino-terminal polypeptide portion corresponding to residues 1 to 220 of glutathione-S-transferase, an intermediate polypeptide portion corresponding to residues 221 tp 226 and defining a cleavage site for the protease Thrombin, a polypeptide portion corresponding to residues 227 to 246 defining a portion of the NANBV capsid antigen that has the amino acid residue sequence 41 to 60 in SEQ ID NO:1, and a carboxy-terminal linker portion corresponding to residues 247 to 252. SEQ ID NO:5 also contains the nucleotide base sequence of a linear single-stranded DNA segment that encodes the fusion protein described therein. The nomenclature and presentation of sequence information is as described for SEQ ID NO:1.

SEQ ID NO:6 contains the linear amino acid residue sequence of a preferred fusion protein designated CAP-A-B and comprised of an amino-terminal polypeptide portion corresponding to residues 1 to 220 of glutathione-S-transferase, an intermediate polypeptide portion corresponding to residues 221 to 226 and defining a cleavage site for the protease Thrombin, a polypeptide portion corresponding to residues 227 to 265 defining a portion of the NANBV capsid antigen that has the amino acid residue sequence 2 to 40 in SEQ ID NO:1, and a carboxy-terminal linker portion corresponding to residues 266 to 271. SEQ ID NO:6 also contains the nucleotide base sequence of a linear single-stranded DNA segment that encodes the fusion protein described therein. The

nomenclature and presentation of sequence information is as described for SEQ ID NO:1.

The foregoing description and the examples are intended as illustrative and are not to be taken as limiting. Still other variations within the spirit and scope of this invention are possible and will readily present themselves to those skilled in the art. Other embodiments are within the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Zebedee, Suzanne
Inchauspe, Genevieve
Nasoff, Marc
Prince, Alfred

(ii) TITLE OF INVENTION: NON-A, NON-B HEPATITIS VIRUS ANTIGEN, DIAGNOSTIC METHODS AND VACCINES

(iii) NUMBER OF SEQUENCES: 137

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: DRESSLER, GOLDSMITH, SHORE, SUTKER & MILNAMOW, LTD.
(B) STREET: 180 N. Stetson, Suite 4700
(C) CITY: Chicago
(D) STATE: IL
(E) COUNTRY: USA
(F) ZIP: 60601

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: US
(B) FILING DATE:
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/616369
(B) FILING DATE: 21-NOV-1990

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: US 07/573643
(B) FILING DATE: 25-AUG-1990

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Gamson, Edward P.
(B) REGISTRATION NUMBER: 29,381
(C) REFERENCE/DOCKET NUMBER: PHA0029P

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 312-616-5400
(B) TELEFAX: 312-616-5460

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 978 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..978
(D) OTHER INFORMATION: /codon_start= 1
/product= "NANBV Structural Antigen"
/number= 1

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

```
ATG AGC ACG ATT CCC AAA CCT CAA AGA AAA ACC AAA CGT AAC ACC AAC      48
Met Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn
 1               5                  10                  15

CGT CGC CCA CAG GAC GTC AAG TTC CCG GGT GGC GGT CAG ATC GTT GGT      96
Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly
            20                  25                  30

GGA GTT TAC TTG TTG CCG CGC AGG GGC CCT AGA TTG GGT GTG CGC GCG     144
Gly Val Tyr Leu Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala
        35                  40                  45

ACG AGG AAG ACT TCC GAG CGG TCG CAA CCT CGA GGT AGA CGT CAG CCT     192
Thr Arg Lys Thr Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro
    50                  55                  60

ATC CCC AAG GCA CGT CGG CCC GAG GGC AGG ACC TGG GCT CAG CCC GGG     240
Ile Pro Lys Ala Arg Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly
65                  70                  75                  80

TAC CCT TGG CCC CTC TAT GGC AAT GAG GGT TGC GGG TGG GCG GGA TGG     288
Tyr Pro Trp Pro Leu Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp
                85                  90                  95

CTC CTG TCT CCC CGT GGC TCT CGG CCT AGC TGG GGC CCC ACA GAC CCC     336
```

```
Leu Leu Ser Pro Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro
          100                 105                 110

CGG CGT AGG TCG CGC AAT TTG GGT AAG GTC ATC GAT ACC CTT ACG TGC      384
Arg Arg Arg Ser Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys
        115                 120                 125

GGC TTC GCC GAC CTC ATG GGG TAC ATA CCG CTC GTC GGC GCC CCT CTT      432
Gly Phe Ala Asp Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu
    130                 135                 140

GGA GGC GCT GCC AGG GCC CTG GCG CAT GGC GTC CGG GTT CTG GAA GAC      480
Gly Gly Ala Ala Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp
145                 150                 155                 160

GGC GTG AAC TAT GCA ACA GGG AAC CTT CCT GGT TGC TCT TTC TCT ATC      528
Gly Val Asn Tyr Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile
                165                 170                 175

TTC CTT CTG GCC CTG CTC TCT TGC CTG ACT GTG CCC GCT TCA GCC TAC      576
Phe Leu Leu Ala Leu Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr
            180                 185                 190

CAA GTG CGC AAT TCC TCG GGG CTT TAC CAT GTC ACC AAT GAT TGC CCT      624
Gln Val Arg Asn Ser Ser Gly Leu Tyr His Val Thr Asn Asp Cys Pro
        195                 200                 205

AAC TCG AGT GTT GTG TAC GAG GCG GCC GAT GCC ATC CTG CAC ACT CCG      672
Asn Ser Ser Val Val Tyr Glu Ala Ala Asp Ala Ile Leu His Thr Pro
    210                 215                 220

GGG TGT GTC CCT TGC GTT CGC GAG GGT AAC GCC TCG AGG TGT TGG GTG      720
Gly Cys Val Pro Cys Val Arg Glu Gly Asn Ala Ser Arg Cys Trp Val
225                 230                 235                 240

GCG GTG ACC CCC ACG GTG GCC ACC AGG GAC GGC AAA CTT CCC ACA ACG      768
Ala Val Thr Pro Thr Val Ala Thr Arg Asp Gly Lys Leu Pro Thr Thr
                245                 250                 255

CAG CTT CGA CGT CAT ATC GAT CTG CTT GTC GGG AGC GCC ACC CTC TGC      816
Gln Leu Arg Arg His Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys
            260                 265                 270

TCG GCC CTC TAC GTG GGG GAC CTG TGC GGG TCT GTC TTT CTC GTT GGT      864
Ser Ala Leu Tyr Val Gly Asp Leu Cys Gly Ser Val Phe Leu Val Gly
        275                 280                 285

CAA CTG TTT ACC TTC TCT CCC AGG CGC CAC TGG ACG ACG CAA GAC TGC      912
Gln Leu Phe Thr Phe Ser Pro Arg Arg His Trp Thr Thr Gln Asp Cys
    290                 295                 300

AAT TGT TCT ATC TAT CCC GGC CAT ATA ACG GGT CAT CGC ATG GCA TGG      960
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```
Asn Cys Ser Ile Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp
305                 310                 315                 320

GAT ATG ATG ATG AAC TGG                                          978
Asp Met Met Met Asn Trp
                325
```

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 948 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..945

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

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ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC      48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
 1               5                  10                  15

ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG      96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG     144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA     192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC     240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA     288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
```

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                    85                  90                  95

GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT      336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA      384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT      432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT      480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA      528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC      576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC      624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG ATC GAA GGT      672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Ile Glu Gly
    210                 215                 220

CGT GGG ATC CCC AAT TCG AGC TCG GTA CCC ATG AGC ACG ATT CCC AAA      720
Arg Gly Ile Pro Asn Ser Ser Ser Val Pro Met Ser Thr Ile Pro Lys
225                 230                 235                 240

CCT CAA AGA AAA ACC AAA CGT AAC ACC AAC CGT CGC CCA CAG GAC GTC      768
Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln Asp Val
                245                 250                 255

AAG TTC CCG GGT GGC GGT CAG ATC GTT GGT GGA GTT TAC TTG TTG CCG      816
Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly Val Tyr Leu Leu Pro
            260                 265                 270

CGC AGG GGC CCT AGA TTG GGT GTG CGC GCG ACG AGG AAG ACT TCC GAG      864
Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser Glu
        275                 280                 285

CGG TCG CAA CCT CGA GGT AGA CGT CAG CCT ATC CCC AAG GCA CGT CGG      912
Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala Arg Arg
```

290 295 300

CCC GAG GGC AGG ACG GGG ATC GGG AAT TCA TCG TGA 948
Pro Glu Gly Arg Thr Gly Ile Gly Asn Ser Ser
305 310 315

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 759 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..756

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC 48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
1 5 10 15

ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG 96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
20 25 30

TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG 144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
35 40 45

GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA 192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
50 55 60

TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC 240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65 70 75 80

ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA 288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
85 90 95

```
GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT      336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA      384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT      432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT      480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA      528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC      576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC      624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT      672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220

GGA TCC ATG AGC ACG ATT CCC AAA CCT CAA AGA AAA ACC AAA CGT AAC      720
Gly Ser Met Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn
225                 230                 235                 240

ACC AAC CGT CGC CCA CAG GAA TTC ATC GTG ACT GAC TGA                  759
Thr Asn Arg Arg Pro Gln Glu Phe Ile Val Thr Asp
                245                 250
```

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 759 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..756

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

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ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC      48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
 1               5                  10                  15

ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG      96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG     144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA     192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC     240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA     288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95

GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT     336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA     384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT     432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT     480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160
```

```
GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA      528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC      576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC      624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT      672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220

GGA TCC GAC GTC AAG TTC CCG GGT GGC GGT CAG ATC GTT GGT GGA GTT      720
Gly Ser Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly Val
225                 230                 235                 240

TAC TTG TTG CCG CGC AGG GAA TTC ATC GTG ACT GAC TGA                  759
Tyr Leu Leu Pro Arg Arg Glu Phe Ile Val Thr Asp
                245                 250
```

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 759 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..756

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC       48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
  1               5                  10                  15

ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG       96
```

```
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
                20                  25                  30

TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG      144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
            35                  40                  45

GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA      192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
        50                  55                  60

TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC      240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
    65                  70                  75                  80

ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA      288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95

GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT      336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA      384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT      432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT      480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA      528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC      576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC      624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT      672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220

GGA TCC GGC CCT AGA TTG GGT GTG CGC GCG ACG AGG AAG ACT TCC GAG      720
```

```
Gly Ser Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr Ser Glu
225                 230                 235                 240

CGG TCG CAA CCT CGA GGT GAA TTC ATC GTG ACT GAC TGA                 759
Arg Ser Gln Pro Arg Gly Glu Phe Ile Val Thr Asp
                245                 250
```

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 816 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..813

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

```
ATG TCC CCT ATA CTA GGT TAT TGG AAA ATT AAG GGC CTT GTG CAA CCC      48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
  1               5                  10                  15

ACT CGA CTT CTT TTG GAA TAT CTT GAA GAA AAA TAT GAA GAG CAT TTG      96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

TAT GAG CGC GAT GAA GGT GAT AAA TGG CGA AAC AAA AAG TTT GAA TTG     144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

GGT TTG GAG TTT CCC AAT CTT CCT TAT TAT ATT GAT GGT GAT GTT AAA     192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

TTA ACA CAG TCT ATG GCC ATC ATA CGT TAT ATA GCT GAC AAG CAC AAC     240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

ATG TTG GGT GGT TGT CCA AAA GAG CGT GCA GAG ATT TCA ATG CTT GAA     288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
```

```
                    85                  90                  95

GGA GCG GTT TTG GAT ATT AGA TAC GGT GTT TCG AGA ATT GCA TAT AGT      336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

AAA GAC TTT GAA ACT CTC AAA GTT GAT TTT CTT AGC AAG CTA CCT GAA      384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

ATG CTG AAA ATG TTC GAA GAT CGT TTA TGT CAT AAA ACA TAT TTA AAT      432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

GGT GAT CAT GTA ACC CAT CCT GAC TTC ATG TTG TAT GAC GCT CTT GAT      480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160

GTT GTT TTA TAC ATG GAC CCA ATG TGC CTG GAT GCG TTC CCA AAA TTA      528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175

GTT TGT TTT AAA AAA CGT ATT GAA GCT ATC CCA CAA ATT GAT AAG TAC      576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

TTG AAA TCC AGC AAG TAT ATA GCA TGG CCT TTG CAG GGC TGG CAA GCC      624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

ACG TTT GGT GGT GGC GAC CAT CCT CCA AAA TCG GAT CTG GTT CCG CGT      672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220

GGA TCC AGC ACG ATT CCC AAA CCT CAA AGA AAA ACC AAA CGT AAC ACC      720
Gly Ser Ser Thr Ile Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr
225                 230                 235                 240

AAC CGT CGC CCA CAG GAC GTC AAG TTC CCG GGT GGC GGT CAG ATC GTT      768
Asn Arg Arg Pro Gln Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val
                245                 250                 255

GGT GGA GTT TAC TTG TTG CCG CGC AGG GAA TTC ATC GTG ACT GAC          813
Gly Gly Val Tyr Leu Leu Pro Arg Arg Glu Phe Ile Val Thr Asp
            260                 265                 270

TGA                                                                  816
```

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

GATCCATGAG CACGATTCCC AAACCTCAAA GAAAAACCAA ACGTAACACC AACCGTCGCC 60

CACAGG 66

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

AATTCCTGTG GGCGACGGTT GGTGTTACGT TTGGTTTTTC TTTGAGGTTT GGGAATCGTG 60

CTCATG 66

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GATCCGACGT CAAGTTCCCG GGTGGCGGTC AGATCGTTGG TGGAGTTTAC TTGTTGCCGC 60

GCAGGG 66

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

AATTCCCTGC GCGGCAACAA GTAAACTCCA CCAACGATCT GACCGCCACC CGGGAACTTG 60

ACGTCG 66

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GATCCGGCCC TAGATTGGGT GTGCGCGCGA CGAGGAAGAC TTCCGAGCGG TCGCAACCTC 60

GAGGTG 66

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 66 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

AATTCACCTC GAGGTTGCGA CCGCTCGGAA GTCTTCCTCG TCGCGCGCAC ACCCAATCTA 60

GGGCCG 66

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

GAATTCTTAC CTGCGCGGCA ACAAGTAAAC TC 32

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 32 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

GCTGGATCCA GCACGATTCC CAAACCTCAA AG 32

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

ATGAGCACGA TTCCCAAACC T 21

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

GAGGAAGACT TCCGAGC 17

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

GTCCTGCCCT CGGGCCG 17

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

ACCCAAATTG CGCGACCTAC G 21

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

TGGGTAAGGT CATCGATAC 19

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

AAGGTCATCG ATACCCT 17

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

AGATAGAGAA AGAGCAAC 18

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

GGACCAGTTC ATCATCATAT AT 22

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

CAGTTCATCA TCATATCCCA 20

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /product= "Linker Protein in GST-NANBV 693-691"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GGG ATC CCC AAT TCA 15
Gly Ile Pro Asn Ser
1 5

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

Gly Ile Pro Asn Ser
1 5

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 12 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..9
(D) OTHER INFORMATION: /product= "Carboxy-terminal Linker Protein in GST-NANBV 693-691"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

AAT TCA TCG TGA 12
Asn Ser Ser
1

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 3 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Asn Ser Ser
1

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /product= "Linker Protein in GST-NANBV 15-18"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

GGG ATC CCC ATC GAA TTC CTG CAG CCC 27
Gly Ile Pro Ile Glu Phe Leu Gln Pro
1 5

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids

(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Gly Ile Pro Ile Glu Phe Leu Gln Pro
1 5

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "Carboxy-terminal Linker Protein in GST-NANBV 15-18"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

TGG GGG ATC GGG AAT TCA TCG TGA 24
Trp Gly Ile Gly Asn Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Trp Gly Ile Gly Asn Ser Ser
1 5

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..24
(D) OTHER INFORMATION: /product= "Linker Protein in GST-NANBV 15-17"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

```
GGG ATC CCC AAT TCC TGC AGC CCT                                    24
Gly Ile Pro Asn Ser Cys Ser Pro
 1               5
```

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 8 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

```
Gly Ile Pro Asn Ser Cys Ser Pro
 1               5
```

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..18
(D) OTHER INFORMATION: /product= "Carboxy-terminal Linker Protein in GST-NANBV 15-17"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

21

```
GGG ATC GGG AAT TCA TCG TGA
Gly Ile Gly Asn Ser Ser
 1               5
```

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

```
Gly Ile Gly Asn Ser Ser
 1               5
```

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS

(B) LOCATION: 1..15
(D) OTHER INFORMATION: /product= "Thrombin Cleavage Site in GST-NANBV 15-17"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

```
GTT CCG CGT GGA TCC                                                    15
Val Pro Arg Gly Ser
  1               5
```

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
Val Pro Arg Gly Ser
  1               5
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "Linker Protein in GST-NANBV 15-17"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
CCA TCG AAT TCC TGC AGC CCT                                            21
Pro Ser Asn Ser Cys Ser Pro
```

1 5

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Pro Ser Asn Ser Cys Ser Pro
1 5

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..15
(D) OTHER INFORMATION: /product= "Carboxy-terminal Linker Protein in GST-NANBV 15-17"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

GGA ATT CAT CGT GAC TGA 18
Gly Ile His Arg Asp
1 5

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Gly Ile His Arg Asp
1 5

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..27
(D) OTHER INFORMATION: /product= "Linker Protein in GST-NANBV 690-691"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

GGG ATC CCC AAT TCG AGC TCG GTA CCC 27
Gly Ile Pro Asn Ser Ser Ser Val Pro
1 5

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Gly Ile Pro Asn Ser Ser Ser Val Pro
1 5

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 1..21
(D) OTHER INFORMATION: /product= "Carboxy-terminal Linker Protein in GST-NANBV 690-691"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

```
ACG GGG ATC GGG AAT TCA TCG TGA                                    24
Thr Gly Ile Gly Asn Ser Ser
 1               5
```

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 7 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: protein

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

```
Thr Gly Ile Gly Asn Ser Ser
 1               5
```

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 9416 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(ix) FEATURE:
(A) NAME/KEY: CDS
(B) LOCATION: 342..9374

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 1..12
(D) OTHER INFORMATION: /note= "Not confirmed as HCV-Hc59 Sequence"

(ix) FEATURE:
(A) NAME/KEY: misc_feature
(B) LOCATION: 9397..9416
(D) OTHER INFORMATION: /note= "Not confirmed as HCV-Hc59 Sequence"

(ix) FEATURE:
(A) NAME/KEY: repeat_unit
(B) LOCATION: group(7..12, 42..47)
(D) OTHER INFORMATION: /rpt_type= "other" /rpt_family= "1"

(ix) FEATURE:
(A) NAME/KEY: repeat_unit
(B) LOCATION: group(23..28, 38..43, 9209..9214, 9391..9396)
(D) OTHER INFORMATION: /rpt_type= "other" /rpt_family= "2"

(ix) FEATURE:
(A) NAME/KEY: repeat_unit
(B) LOCATION: group(128..135, 315..322)
(D) OTHER INFORMATION: /rpt_type= "other" /rpt_family= "3"

(ix) FEATURE:
(A) NAME/KEY: repeat_unit
(B) LOCATION: group(9231..9237, 9245..9251, 9256..9262)
(D) OTHER INFORMATION: /rpt_type= "other" /rpt_family= "4"

(ix) FEATURE:
(A) NAME/KEY: repeat_unit
(B) LOCATION: group(9248..9253, 9221..9226, 9227..9232)
(D) OTHER INFORMATION: /rpt_type= "other" /rpt_family= "5"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

```
GCCAGCCCCC TGATGGGGGC GACACTCCAC CATGAATCAC TCCCCTGTGA GGAACTACTG      60

TCTTCACGCA GAAAGCGTCT AGCCATGGCG TTAGTATGAG TGTCGTGCAG CCTCCAGGAC     120

CCCCCCTCCC GGGAGAGCCA TAGTGGTCTG CGGAACCGGT GAGTACACCG GAATTGCCAG     180

GACGACCGGG TCCTTTCTTG GATAAACCCG CTCAATGCCT GGAGATTTGG GCGTGCCCCC     240

GCAAGACTGC TAGCCGAGTA GTGTTGGGTC GCGAAAGGCC TTGTGGTACT GCCTGATAGG     300

GTGCTTGCGA GTGCCCCGGG AGGTCTCGTA GACCGTGCAC C ATG AGC ACG AAT          353
                                               Met Ser Thr Asn
                                                 1

CCT AAA CCT CAA AGA AAA ACC AAA CGT AAC ACC AAC CGT CGC CCA CAG       401
Pro Lys Pro Gln Arg Lys Thr Lys Arg Asn Thr Asn Arg Arg Pro Gln
  5                  10                  15                  20

GAC GTC AAG TTC CCG GGT GGC GGT CAG ATC GTT GGT GGA GTT TAC TTG       449
Asp Val Lys Phe Pro Gly Gly Gly Gln Ile Val Gly Gly Val Tyr Leu
                  25                  30                  35

TTG CCG CGC AGG GGC CCT AGA TTG GGT GTG CGC GCG ACG AGG AAG ACT       497
Leu Pro Arg Arg Gly Pro Arg Leu Gly Val Arg Ala Thr Arg Lys Thr
             40                  45                  50

TCC GAG CGG TCG CAA CCT CGA GGT AGA CGT CAG CCT ATC CCC AAG GCA       545
Ser Glu Arg Ser Gln Pro Arg Gly Arg Arg Gln Pro Ile Pro Lys Ala
         55                  60                  65

CGT CGG CCC GAG GGC AGG ACC TGG GCT CAG CCC GGG TAC CCT TGG CCC       593
Arg Arg Pro Glu Gly Arg Thr Trp Ala Gln Pro Gly Tyr Pro Trp Pro
     70                  75                  80

CTC TAT GGC AAT GAG GGT TGC GGG TGG GCG GGA TGG CTC CTG TCT CCC       641
Leu Tyr Gly Asn Glu Gly Cys Gly Trp Ala Gly Trp Leu Leu Ser Pro
 85                  90                  95                 100

CGT GGC TCT CGG CCT AGC TGG GGC CCC ACA GAC CCC CGG CGT AGG TCG       689
Arg Gly Ser Arg Pro Ser Trp Gly Pro Thr Asp Pro Arg Arg Arg Ser
                 105                 110                 115

CGC AAT TTG GGT AAG GTC ATC GAT ACC CTT ACG TGC GGC TTC GCC GAC       737
Arg Asn Leu Gly Lys Val Ile Asp Thr Leu Thr Cys Gly Phe Ala Asp
             120                 125                 130

CTC ATG GGG TAC ATA CCG CTC GTC GGC GCC CCT CTT GGA GGC GCT GCC       785
Leu Met Gly Tyr Ile Pro Leu Val Gly Ala Pro Leu Gly Gly Ala Ala
         135                 140                 145
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AGG GCC CTG GCG CAT GGC GTC CGG GTT CTG GAA GAC GGC GTG AAC TAT          833
Arg Ala Leu Ala His Gly Val Arg Val Leu Glu Asp Gly Val Asn Tyr
    150                 155                 160

GCA ACA GGG AAC CTT CCT GGT TGC TCT TTC TCT ATC TTC CTT CTG GCC          881
Ala Thr Gly Asn Leu Pro Gly Cys Ser Phe Ser Ile Phe Leu Leu Ala
165                 170                 175                 180

CTG CTC TCT TGC CTG ACT GTG CCC GCT TCA GCC TAC CAA GTG CGC AAT          929
Leu Leu Ser Cys Leu Thr Val Pro Ala Ser Ala Tyr Gln Val Arg Asn
                185                 190                 195

TCC TCG GGG CTT TAC CAT GTC ACC AAT GAT TGC CCT AAC TCG AGT GTT          977
Ser Ser Gly Leu Tyr His Val Thr Asn Asp Cys Pro Asn Ser Ser Val
            200                 205                 210

GTG TAC GAG GCG GCC GAT GCC ATC CTG CAC ACT CCG GGG TGT GTC CCT         1025
Val Tyr Glu Ala Ala Asp Ala Ile Leu His Thr Pro Gly Cys Val Pro
        215                 220                 225

TGC GTT CGC GAG GGT AAC GCC TCG AGG TGT TGG GTG GCG GTG ACC CCC         1073
Cys Val Arg Glu Gly Asn Ala Ser Arg Cys Trp Val Ala Val Thr Pro
    230                 235                 240

ACG GTG GCC ACC AGG GAC GGC AAA CTC CCC ACA ACG CAG CTT CGA CGT         1121
Thr Val Ala Thr Arg Asp Gly Lys Leu Pro Thr Thr Gln Leu Arg Arg
245                 250                 255                 260

CAT ATC GAT CTG CTT GTC GGG AGC GCC ACC CTC TGC TCG GCC CTC TAC         1169
His Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu Tyr
                265                 270                 275

GTG GGG GAC CTG TGC GGG TCT GTC TTT CTT GTT GGT CAA CTG TTT ACC         1217
Val Gly Asp Leu Cys Gly Ser Val Phe Leu Val Gly Gln Leu Phe Thr
            280                 285                 290

TTC TCT CCC AGG CAC CAC TGG ACG ACG CAA GAC TGC AAT TGT TCT ATC         1265
Phe Ser Pro Arg His His Trp Thr Thr Gln Asp Cys Asn Cys Ser Ile
        295                 300                 305

TAT CCC GGC CAT ATA ACG GGT CAT CGC ATG GCA TGG AAT ATG ATG ATG         1313
Tyr Pro Gly His Ile Thr Gly His Arg Met Ala Trp Asn Met Met Met
    310                 315                 320

AAC TGG TCC CCT ACG GCA GCG TTG GTG GTA GCT CAG CTG CTC CGA ATC         1361
Asn Trp Ser Pro Thr Ala Ala Leu Val Val Ala Gln Leu Leu Arg Ile
325                 330                 335                 340

CCA CAA GCC ATC ATG GAC ATG ATC GCT GGC GCC CAC TGG GGA GTC CTG         1409
Pro Gln Ala Ile Met Asp Met Ile Ala Gly Ala His Trp Gly Val Leu
                345                 350                 355
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GCG GGC ATA AAG TAT TTC TCC ATG GTG GGG AAC TGG GCG AAG GTC CTG      1457
Ala Gly Ile Lys Tyr Phe Ser Met Val Gly Asn Trp Ala Lys Val Leu
            360                 365                 370

GTA GTG CTG CTG CTA TTT GCC GGC GTC GAC GCG GAA ACC CAC GTC ACC      1505
Val Val Leu Leu Leu Phe Ala Gly Val Asp Ala Glu Thr His Val Thr
        375                 380                 385

GGG GGA AAT GCC GGC CGC ACC ACG GCT GGG CTT GTT GGT CTC CTT ACA      1553
Gly Gly Asn Ala Gly Arg Thr Thr Ala Gly Leu Val Gly Leu Leu Thr
    390                 395                 400

CCA GGC GCC AAG CAG AAC ATC CAA CTG ATC AAC ACC AAC GGC AGT TGG      1601
Pro Gly Ala Lys Gln Asn Ile Gln Leu Ile Asn Thr Asn Gly Ser Trp
405                 410                 415                 420

CAC ATC AAT AGC ACG GCC TTG AAC TGC AAT GAA AGC CTT AAC ACC GGC      1649
His Ile Asn Ser Thr Ala Leu Asn Cys Asn Glu Ser Leu Asn Thr Gly
                425                 430                 435

TGG TTA GCA GGG CTC TTC TAT CAG CAC AAA TTC AAC TCT TCA GGC TGT      1697
Trp Leu Ala Gly Leu Phe Tyr Gln His Lys Phe Asn Ser Ser Gly Cys
            440                 445                 450

CCT GAG AGG TTG GCC AGC TGC CGA CGC CTT ACC GAT TTT GCC CAG GGC      1745
Pro Glu Arg Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Ala Gln Gly
        455                 460                 465

TGG GGT CCT ATC AGT TAT GCC AAC GGA AGC GGC CTC GAC GAA CGC CCC      1793
Trp Gly Pro Ile Ser Tyr Ala Asn Gly Ser Gly Leu Asp Glu Arg Pro
    470                 475                 480

TAC TGC TGG CAC TAC CCT CCA AGA CCT TGT GGC ATT GTG CCC GCA AAG      1841
Tyr Cys Trp His Tyr Pro Pro Arg Pro Cys Gly Ile Val Pro Ala Lys
485                 490                 495                 500

AGC GTG TGT GGC CCG GTA TAT TGC TTC ACT CCC AGC CCC GTG GTG GTG      1889
Ser Val Cys Gly Pro Val Tyr Cys Phe Thr Pro Ser Pro Val Val Val
                505                 510                 515

GGA ACG ACC GAC AGG TCG GGC GCG CCT ACC TAC AGC TGG GGT GCA AAT      1937
Gly Thr Thr Asp Arg Ser Gly Ala Pro Thr Tyr Ser Trp Gly Ala Asn
            520                 525                 530

GAT ACG GAT GTC TTC GTC CTT AAC AAC ACC AGG CCA CCG CTG GGC AAT      1985
Asp Thr Asp Val Phe Val Leu Asn Asn Thr Arg Pro Pro Leu Gly Asn
        535                 540                 545

TGG TTC GGT TGT ACC TGG ATG AAC TCA ACT GGA TTC ACC AAA GTG TGC      2033
Trp Phe Gly Cys Thr Trp Met Asn Ser Thr Gly Phe Thr Lys Val Cys
    550                 555                 560
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GGA GCG CCC CCT TGT GTC ATC GGA GGG GTG GGC AAC AAC ACC TTG CTC      2081
Gly Ala Pro Pro Cys Val Ile Gly Gly Val Gly Asn Asn Thr Leu Leu
565                 570                 575                 580

TGC CCC ACT GAT TGC TTC CGC AAA TAT CCG GAA GCC ACA TAC TCT CGG      2129
Cys Pro Thr Asp Cys Phe Arg Lys Tyr Pro Glu Ala Thr Tyr Ser Arg
                585                 590                 595

TGC GGC TCC GGT CCC AGG ATT ACA CCC AGG TGC ATG GTC GAC TAC CCG      2177
Cys Gly Ser Gly Pro Arg Ile Thr Pro Arg Cys Met Val Asp Tyr Pro
            600                 605                 610

TAT AGG CTT TGG CAC TAT CCT TGT ACC ATC AAT TAC ACC ATA TTC AAA      2225
Tyr Arg Leu Trp His Tyr Pro Cys Thr Ile Asn Tyr Thr Ile Phe Lys
        615                 620                 625

GTC AGG ATG TAC GTG GGA GGG GTC GAG CAC AGG CTG GAA GCG GCC TGC      2273
Val Arg Met Tyr Val Gly Gly Val Glu His Arg Leu Glu Ala Ala Cys
    630                 635                 640

AAC TGG ACG CGG GGC GAA CGC TGT GAT CTG GAA GAC AGG GAC AGG TCC      2321
Asn Trp Thr Arg Gly Glu Arg Cys Asp Leu Glu Asp Arg Asp Arg Ser
645                 650                 655                 660

GAG CTC AGC CCG TTG CTG CTG TCC ACC ACA CAG TGG CAG GTC CTT CCG      2369
Glu Leu Ser Pro Leu Leu Leu Ser Thr Thr Gln Trp Gln Val Leu Pro
                665                 670                 675

TGT TCT TTC ACG ACC CTG CCA GCC TTG TCC ACC GGC CTC ATC CAC CTC      2417
Cys Ser Phe Thr Thr Leu Pro Ala Leu Ser Thr Gly Leu Ile His Leu
            680                 685                 690

CAC CAG AAC ATT GTG GAC GTG CAG TAC TTG TAC GGG GTA GGG TCA AGC      2465
His Gln Asn Ile Val Asp Val Gln Tyr Leu Tyr Gly Val Gly Ser Ser
        695                 700                 705

ATC GCG TCC TGG GCC ATT AAG TGG GAG TAC GTC GTT CTC CTG TTC CTT      2513
Ile Ala Ser Trp Ala Ile Lys Trp Glu Tyr Val Val Leu Leu Phe Leu
    710                 715                 720

CTG CTT GCA GAC GCG CGC GTC TGT TCC TGC TTG TGG ATG ATG TTA CTC      2561
Leu Leu Ala Asp Ala Arg Val Cys Ser Cys Leu Trp Met Met Leu Leu
725                 730                 735                 740

ATA TCC CAA GCG GAG GCG GCT TTG GAG AAC CTC GTA ATA CTC AAT GCA      2609
Ile Ser Gln Ala Glu Ala Ala Leu Glu Asn Leu Val Ile Leu Asn Ala
                745                 750                 755

GCA TCC CTG GCC GGG ACG CAT GGT CTT GTG TCC TTC CTC GTG TTC TTC      2657
Ala Ser Leu Ala Gly Thr His Gly Leu Val Ser Phe Leu Val Phe Phe
            760                 765                 770
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TGC TTT GCG TGG TAT CTG AAG GGT AGG TGG GTG CCC GGA GCG GTC TAC      2705
Cys Phe Ala Trp Tyr Leu Lys Gly Arg Trp Val Pro Gly Ala Val Tyr
        775                 780                 785

GCC CTC TAC GGG ATG TGG CCT CTC CTC CTG CTC CTG CTG GCG TTG CCT      2753
Ala Leu Tyr Gly Met Trp Pro Leu Leu Leu Leu Leu Leu Ala Leu Pro
    790                 795                 800

CAG CGG GCA TAC GCA CTG GAC ACG GAG GTG GCC GCG TCG TGT GGC GGC      2801
Gln Arg Ala Tyr Ala Leu Asp Thr Glu Val Ala Ala Ser Cys Gly Gly
805                 810                 815                 820

GTT GTT CTT GTC GGG TTA ATG GCG CTG ACT CTG TCG CCA TAT TAC AAG      2849
Val Val Leu Val Gly Leu Met Ala Leu Thr Leu Ser Pro Tyr Tyr Lys
                825                 830                 835

CGC TAT ATC AGC TGG TGC ATG TGG TGG CTT CAG TAT TTT CTG ACC AGA      2897
Arg Tyr Ile Ser Trp Cys Met Trp Trp Leu Gln Tyr Phe Leu Thr Arg
            840                 845                 850

GTA GAA GCG CAA CTG CAC GTG TGG GTT CCC CCC CTC AAC GTC CGG GGG      2945
Val Glu Ala Gln Leu His Val Trp Val Pro Pro Leu Asn Val Arg Gly
        855                 860                 865

GGG CGC GAT GCC GTC ATC TTA CTC ACG TGT GTA GTA CAC CCG GCC CTG      2993
Gly Arg Asp Ala Val Ile Leu Leu Thr Cys Val Val His Pro Ala Leu
    870                 875                 880

GTA TTT GAC ATC ACC AAA CTA CTC CTG GCC ATC TTC GGA CCC CTT TGG      3041
Val Phe Asp Ile Thr Lys Leu Leu Leu Ala Ile Phe Gly Pro Leu Trp
885                 890                 895                 900

ATT CTT CAA GCC AGT TTG CTT AAA GTC CCC TAC TTC GTG CGC GTT CAA      3089
Ile Leu Gln Ala Ser Leu Leu Lys Val Pro Tyr Phe Val Arg Val Gln
                905                 910                 915

GGC CTT CTC CGG ATC TGC GCG CTA GCG CGG AAG ATA GCC GGA GGT CAT      3137
Gly Leu Leu Arg Ile Cys Ala Leu Ala Arg Lys Ile Ala Gly Gly His
            920                 925                 930

TAC GTG CAA ATG GCC ATC ATC AAG TTA GGG GCG CTT ACT GGC ACC TGT      3185
Tyr Val Gln Met Ala Ile Ile Lys Leu Gly Ala Leu Thr Gly Thr Cys
        935                 940                 945

GTG TAT AAC CAT CTC GCT CCT CTT CGA GAC TGG GCG CAC AAC GGC CTG      3233
Val Tyr Asn His Leu Ala Pro Leu Arg Asp Trp Ala His Asn Gly Leu
    950                 955                 960

CGA GAT CTG GCC GTG GCT GTG GAA CCA GTC GTC TTC TCC CGA ATG GAG      3281
Arg Asp Leu Ala Val Ala Val Glu Pro Val Val Phe Ser Arg Met Glu
965                 970                 975                 980
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ACC AAG CTC ATC ACG TGG GGG GCA GAT ACC GCC GCG TGC GGT GAC ATC      3329
Thr Lys Leu Ile Thr Trp Gly Ala Asp Thr Ala Ala Cys Gly Asp Ile
                985                 990                 995

ATC AAC GGC TTG CCC GTC TCT GCC CGT AGG GGC CAG GAG ATA CTG CTT      3377
Ile Asn Gly Leu Pro Val Ser Ala Arg Arg Gly Gln Glu Ile Leu Leu
           1000                1005                1010

GGG CCA GCC GAC GGA ATG GTC TCC AAG GGG TGG AGG TTG CTG GCG CCC      3425
Gly Pro Ala Asp Gly Met Val Ser Lys Gly Trp Arg Leu Leu Ala Pro
      1015                1020                1025

ATC ACG GCG TAC GCC CAG CAG ACG AGA GGC CTC CTA GGG TGT ATA ATC      3473
Ile Thr Ala Tyr Ala Gln Gln Thr Arg Gly Leu Leu Gly Cys Ile Ile
 1030                1035                1040

ACC AGC CTG ACT GGC CGG GAC AAA AAC CAA GTG GAG GGT GAG GTC CAG      3521
Thr Ser Leu Thr Gly Arg Asp Lys Asn Gln Val Glu Gly Glu Val Gln
1045                1050                1055                1060

ATC GTG TCA ACT GCT ACC CAG ACC TTC CTG GCA ACG TGC ATC AAT GGG      3569
Ile Val Ser Thr Ala Thr Gln Thr Phe Leu Ala Thr Cys Ile Asn Gly
                1065                1070                1075

GTA TGC TGG ACT GTC TAC CAC GGG GCC GGA ACG AGG ACC ATC GCA TCA      3617
Val Cys Trp Thr Val Tyr His Gly Ala Gly Thr Arg Thr Ile Ala Ser
           1080                1085                1090

CCC AAG GGT CCT GTC ATC CAG ACG TAT ACC AAT GTG GAT CAA GAC CTC      3665
Pro Lys Gly Pro Val Ile Gln Thr Tyr Thr Asn Val Asp Gln Asp Leu
      1095                1100                1105

GTG GGC TGG CCC GCT CCT CAA GGT TCC CGC TCA TTG ACA CCC TGC ACC      3713
Val Gly Trp Pro Ala Pro Gln Gly Ser Arg Ser Leu Thr Pro Cys Thr
 1110                1115                1120

TGC GGC TCC TCG GAC CTT TAC CTG GTC ACG AGG CAC GCC GAT GTC ATT      3761
Cys Gly Ser Ser Asp Leu Tyr Leu Val Thr Arg His Ala Asp Val Ile
1125                1130                1135                1140

CCC GTG CGC CGG CGA GGT GAT AGC AGG GGT AGC CTG CTT TCG CCC CGG      3809
Pro Val Arg Arg Arg Gly Asp Ser Arg Gly Ser Leu Leu Ser Pro Arg
                1145                1150                1155

CCC ATT TCC TAC TTG AAA GGC TCC TCG GGG GGT CCG CTG TTG TGC CCC      3857
Pro Ile Ser Tyr Leu Lys Gly Ser Ser Gly Gly Pro Leu Leu Cys Pro
           1160                1165                1170

ACG GGA CAC GCC GTG GGC CTA TTC AGG GCC GCG GTG TGC ACC CGT GGA      3905
Thr Gly His Ala Val Gly Leu Phe Arg Ala Ala Val Cys Thr Arg Gly
      1175                1180                1185
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GTG GCT AAG GCG GTG GAC TTT ATC CCT GTG GAG AAC CTA GAG ACA ACC      3953
Val Ala Lys Ala Val Asp Phe Ile Pro Val Glu Asn Leu Glu Thr Thr
    1190                1195                1200

ATG AGA TCC CCG GTG TTC ACG GAC AAC TCC TCT CCA CCA GCA GTG CCC      4001
Met Arg Ser Pro Val Phe Thr Asp Asn Ser Ser Pro Pro Ala Val Pro
1205                1210                1215                1220

CAG AGC TTC CAG GTG GCC CAC CTG CAT GCT CCC ACC GGC AGC GGT AAG      4049
Gln Ser Phe Gln Val Ala His Leu His Ala Pro Thr Gly Ser Gly Lys
                1225                1230                1235

AGC ACC AAG GTC CCG GCT GCG TAC GCA GCC AAG GGC TAC AAG GTG TTG      4097
Ser Thr Lys Val Pro Ala Ala Tyr Ala Ala Lys Gly Tyr Lys Val Leu
            1240                1245                1250

GTG CTC AAC CCC TCT GTT GCT GCA ACA CTG GGC TTT GGT GCT TAC ATG      4145
Val Leu Asn Pro Ser Val Ala Ala Thr Leu Gly Phe Gly Ala Tyr Met
        1255                1260                1265

TCC AAG GCC CAT GGG GTT GAT CCT AAT ATC AGG ACC GGG GTG AGA ACA      4193
Ser Lys Ala His Gly Val Asp Pro Asn Ile Arg Thr Gly Val Arg Thr
    1270                1275                1280

ATT ACC ACT GGC AGC CCC ATC ACG TAC TCC ACC TAC GGC AAG TTC CTT      4241
Ile Thr Thr Gly Ser Pro Ile Thr Tyr Ser Thr Tyr Gly Lys Phe Leu
1285                1290                1295                1300

GCC GAC GCC GGG TGC TCA GGA GGT GCT TAT GAC ATA ATA ATT TGT GAC      4289
Ala Asp Ala Gly Cys Ser Gly Gly Ala Tyr Asp Ile Ile Ile Cys Asp
                1305                1310                1315

GAG TGC CAC TCC ACG GAT GCC ACA TCC ATC TCG GGC ATC GGC ACT GTC      4337
Glu Cys His Ser Thr Asp Ala Thr Ser Ile Ser Gly Ile Gly Thr Val
            1320                1325                1330

CTT GAC CAA GCA GAG ACT GCG GGG GCG AGA CTG GTT GTG CTC GCC ACT      4385
Leu Asp Gln Ala Glu Thr Ala Gly Ala Arg Leu Val Val Leu Ala Thr
        1335                1340                1345

GCT ACC CCT CCG GGC TCC GTC ACT GTG TCC CAT CCT AAC ATC GAG GAG      4433
Ala Thr Pro Pro Gly Ser Val Thr Val Ser His Pro Asn Ile Glu Glu
    1350                1355                1360

GTT GCT CTG TCC ACC ACC GGA GAG ATC CCC TTT TAC GGC AAG GCT ATC      4481
Val Ala Leu Ser Thr Thr Gly Glu Ile Pro Phe Tyr Gly Lys Ala Ile
1365                1370                1375                1380

CCC CTC GAG GTG ATC AAG GGG GGA AGA CAT CTC ATC TTC TGC CAC TCA      4529
Pro Leu Glu Val Ile Lys Gly Gly Arg His Leu Ile Phe Cys His Ser
                1385                1390                1395
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AAG AAG AAG TGC GAC GAG CTC GCC GCG AAG CTG GTC GCA TTG GGC ATC      4577
Lys Lys Lys Cys Asp Glu Leu Ala Ala Lys Leu Val Ala Leu Gly Ile
            1400                1405                1410

AAT GCC GTG GCC TAC TAC CGC GGT CTT GAC GTG TCT GTC ATC CCG ACC      4625
Asn Ala Val Ala Tyr Tyr Arg Gly Leu Asp Val Ser Val Ile Pro Thr
        1415                1420                1425

AGC GGC GAT GTT GTC GTC GTG TCG ACC GAT GCT CTC ATG ACT GGC TTT      4673
Ser Gly Asp Val Val Val Val Ser Thr Asp Ala Leu Met Thr Gly Phe
    1430                1435                1440

ACC GGC GAC TTC GAC TCT GTG ATA GAC TGC AAC ACG TGT GTC ACT CAG      4721
Thr Gly Asp Phe Asp Ser Val Ile Asp Cys Asn Thr Cys Val Thr Gln
1445                1450                1455                1460

ACA GTC GAT TTT AGC CTT GAC CCT ACC TTT ACC ATT GAG ACA ACC ACG      4769
Thr Val Asp Phe Ser Leu Asp Pro Thr Phe Thr Ile Glu Thr Thr Thr
                1465                1470                1475

CTC CCC CAG GAT GCT GTC TCC AGG ACT CAA CGC CGG GGC AGG ACT GGC      4817
Leu Pro Gln Asp Ala Val Ser Arg Thr Gln Arg Arg Gly Arg Thr Gly
            1480                1485                1490

AGG GGG AAG CCA GGC ATC TAT AGA TTT GTG GCA CCG GGG GAG CGC CCC      4865
Arg Gly Lys Pro Gly Ile Tyr Arg Phe Val Ala Pro Gly Glu Arg Pro
        1495                1500                1505

TCC GGC ATG TTC GAC TCG TCC GTC CTC TGT GAG TGC TAT GAC GCG GGC      4913
Ser Gly Met Phe Asp Ser Ser Val Leu Cys Glu Cys Tyr Asp Ala Gly
    1510                1515                1520

TGT GCT TGG TAT GAG CTC ACG CCC GCC GAG ACT ACA GTT AGG CTA CGA      4961
Cys Ala Trp Tyr Glu Leu Thr Pro Ala Glu Thr Thr Val Arg Leu Arg
1525                1530                1535                1540

GCG TAC ATG AAC ACC CCG GGG CTT CCC GTG TGC CAG GAC CAT CTT GGA      5009
Ala Tyr Met Asn Thr Pro Gly Leu Pro Val Cys Gln Asp His Leu Gly
                1545                1550                1555

TTT TGG GAG GGC GTC TTT ACG GGC CTC ACT CAT ATA GAT GCC CAC TTT      5057
Phe Trp Glu Gly Val Phe Thr Gly Leu Thr His Ile Asp Ala His Phe
            1560                1565                1570

CTA TCC CAG ACA AAG CAG AGT GGG GAG AAC TTT CCT TAC CTG GTA GCG      5105
Leu Ser Gln Thr Lys Gln Ser Gly Glu Asn Phe Pro Tyr Leu Val Ala
        1575                1580                1585

TAC CAA GCC ACC GTG TGC GCT AGG GCT CAA GCC CCT CCC CCA TCG TGG      5153
Tyr Gln Ala Thr Val Cys Ala Arg Ala Gln Ala Pro Pro Pro Ser Trp
    1590                1595                1600
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GAC CAG ATG CGG AAG TGT TTG ATC CGC CTT AAA CCC ACC CTC CAT GGG      5201
Asp Gln Met Arg Lys Cys Leu Ile Arg Leu Lys Pro Thr Leu His Gly
1605                1610                1615                1620

CCA ACA CCC CTG CTA TAC AGA CTG GGC GCT GTT CAG AAT GAA GTC ACC      5249
Pro Thr Pro Leu Leu Tyr Arg Leu Gly Ala Val Gln Asn Glu Val Thr
                    1625                1630                1635

CTG ACG CAC CCA ATC ACC AAA TAC ATC ATG ACA TGC ATG TCG GCC GAC      5297
Leu Thr His Pro Ile Thr Lys Tyr Ile Met Thr Cys Met Ser Ala Asp
            1640                1645                1650

CTG GAG GTC GTC ACG AGC ACC TGG GTG CTC GTT GGC GGC GTC CTG GCT      5345
Leu Glu Val Val Thr Ser Thr Trp Val Leu Val Gly Gly Val Leu Ala
        1655                1660                1665

GCT CTG GCC GCG TAT TGC CTG TCA ACA GGC TGC GTG GTC ATA GTG GGC      5393
Ala Leu Ala Ala Tyr Cys Leu Ser Thr Gly Cys Val Val Ile Val Gly
    1670                1675                1680

AGG ATC GTC TTG TCC GGG AAG CCG GCA ATT ATA CCT GAC AGG GAG GTT      5441
Arg Ile Val Leu Ser Gly Lys Pro Ala Ile Ile Pro Asp Arg Glu Val
1685                1690                1695                1700

CTC TAC CAG GAG TTC GAT GAG ATG GAA GAG TGC TCT CAG CAC TTA CCG      5489
Leu Tyr Gln Glu Phe Asp Glu Met Glu Glu Cys Ser Gln His Leu Pro
                    1705                1710                1715

TAC ATC GAG CAA GGG ATG ATG CTC GCT GAG CAG TTC AAG CAG AAG GCC      5537
Tyr Ile Glu Gln Gly Met Met Leu Ala Glu Gln Phe Lys Gln Lys Ala
            1720                1725                1730

CTC GGC CTC CTG CAG ACC GCG TCC CGC CAT GCA GAG GTT ATC ACC CCT      5585
Leu Gly Leu Leu Gln Thr Ala Ser Arg His Ala Glu Val Ile Thr Pro
        1735                1740                1745

GCT GTC CAG ACC AAC TGG CAG AAA CTC GAG GTC TTT TGG GCG AAG CAC      5633
Ala Val Gln Thr Asn Trp Gln Lys Leu Glu Val Phe Trp Ala Lys His
    1750                1755                1760

ATG TGG AAT TTC ATC AGT GGG ATA CAA TAC TTG GCG GGC CTG TCA ACG      5681
Met Trp Asn Phe Ile Ser Gly Ile Gln Tyr Leu Ala Gly Leu Ser Thr
1765                1770                1775                1780

CTG CCT GGT AAC CCC GCC ATT GCT TCA TTG ATG GCT TTT ACA GCT GCC      5729
Leu Pro Gly Asn Pro Ala Ile Ala Ser Leu Met Ala Phe Thr Ala Ala
                    1785                1790                1795

GTC ACC AGC CCA CTA ACC ACT GGC CAA ACC CTC CTC TTC AAC ATA TTG      5777
Val Thr Ser Pro Leu Thr Thr Gly Gln Thr Leu Leu Phe Asn Ile Leu
            1800                1805                1810
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GGG GGG TGG GTG GCT GCC CAG CTC GCC GCC CCC GGT GCC GCT ACC GCC      5825
Gly Gly Trp Val Ala Ala Gln Leu Ala Ala Pro Gly Ala Ala Thr Ala
        1815                1820                1825

TTT GTG GGC GCT GGC TTA GCT GGC GCC GCA CTC GAC AGC GTT GGA CTG      5873
Phe Val Gly Ala Gly Leu Ala Gly Ala Ala Leu Asp Ser Val Gly Leu
    1830                1835                1840

GGG AAG GTC CTC GTG GAC ATT CTT GCA GGC TAT GGC GCG GGC GTG GCG      5921
Gly Lys Val Leu Val Asp Ile Leu Ala Gly Tyr Gly Ala Gly Val Ala
1845                1850                1855                1860

GGA GCT CTT GTG GCA TTC AAG ATC ATG AGC GGT GAG GTC CCC TCC ACG      5969
Gly Ala Leu Val Ala Phe Lys Ile Met Ser Gly Glu Val Pro Ser Thr
                1865                1870                1875

GAG GAC CTG GTC AAT CTG CTG CCC GCC ATC CTC TCA CCT GGA GCC CTT      6017
Glu Asp Leu Val Asn Leu Leu Pro Ala Ile Leu Ser Pro Gly Ala Leu
            1880                1885                1890

GCA GTC GGT GTG GTC TTT GCA TCA ATA CTG CGC CGG CGT GTT GGC CCG      6065
Ala Val Gly Val Val Phe Ala Ser Ile Leu Arg Arg Arg Val Gly Pro
        1895                1900                1905

GGC GAG GGG GCA GTG CAA TGG ATG AAC CGG CTA ATA GCC TTC GCC TCC      6113
Gly Glu Gly Ala Val Gln Trp Met Asn Arg Leu Ile Ala Phe Ala Ser
    1910                1915                1920

CGG GGG AAC CAT GTT TCC CCC ACA CAC TAC GTG CCG GAG AGC GAT GCA      6161
Arg Gly Asn His Val Ser Pro Thr His Tyr Val Pro Glu Ser Asp Ala
1925                1930                1935                1940

GCC GCC CGC GTC ACT GCC ATA CTC AGC AGC CTC ACT GTA ACC CAG CTC      6209
Ala Ala Arg Val Thr Ala Ile Leu Ser Ser Leu Thr Val Thr Gln Leu
                1945                1950                1955

CTG AGG CGA CTG CAT CAG TGG ATA AGC TCG GAG TGT ACC ACT CCA TGC      6257
Leu Arg Arg Leu His Gln Trp Ile Ser Ser Glu Cys Thr Thr Pro Cys
            1960                1965                1970

TCC GGT TCC TGG CTA AGG GAC ATC TGG GAC TGG ATA TGC GAG GTG CTG      6305
Ser Gly Ser Trp Leu Arg Asp Ile Trp Asp Trp Ile Cys Glu Val Leu
        1975                1980                1985

AGC GAC TTT AAG ACC TGG CTG AAA GCC AAG CTC ATG CCA CAA CTG CCT      6353
Ser Asp Phe Lys Thr Trp Leu Lys Ala Lys Leu Met Pro Gln Leu Pro
    1990                1995                2000

GGG ATT CCC TTT GTG TCC TGC CAG CGC GGG TAT AGG GGG GTC TGG CGA      6401
Gly Ile Pro Phe Val Ser Cys Gln Arg Gly Tyr Arg Gly Val Trp Arg
2005                2010                2015                2020
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GGA GAC GGC ATT ATG CAC ACT CGC TGC CAC TGT GGA GCT GAG ATC ACT      6449
Gly Asp Gly Ile Met His Thr Arg Cys His Cys Gly Ala Glu Ile Thr
            2025                2030                2035

GGA CAT GTC AAA AAC GGG ACG ATG AGG ATC GTC GGT CCT AGG ACC TGC      6497
Gly His Val Lys Asn Gly Thr Met Arg Ile Val Gly Pro Arg Thr Cys
        2040                2045                2050

AAG AAC ATG TGG AGT GGG ACG TTC TTC ATT AAT GCC TAC ACC ACG GGC      6545
Lys Asn Met Trp Ser Gly Thr Phe Phe Ile Asn Ala Tyr Thr Thr Gly
    2055                2060                2065

CCC TGT ACT CCC CTT CCT GCG CCG AAC TAT AAG TTC GCG CTG TGG AGG      6593
Pro Cys Thr Pro Leu Pro Ala Pro Asn Tyr Lys Phe Ala Leu Trp Arg
2070                2075                2080

GTG TCT GCA GAG GAA TAC GTG GAG ATA AGG CGG GTG GGG GAC TTC CAC      6641
Val Ser Ala Glu Glu Tyr Val Glu Ile Arg Arg Val Gly Asp Phe His
2085                2090                2095                2100

TAC GTA TCG GGC ATG ACT ACT GAC AAT CTC AAA TGC CCG TGC CAG ATC      6689
Tyr Val Ser Gly Met Thr Thr Asp Asn Leu Lys Cys Pro Cys Gln Ile
            2105                2110                2115

CCA TCG CCC GAA TTT TTC ACA GAA TTG GAC GGG GTG CGC CTA CAT AGG      6737
Pro Ser Pro Glu Phe Phe Thr Glu Leu Asp Gly Val Arg Leu His Arg
        2120                2125                2130

TTT GCG CCC CCT TGC AAG CCC TTG CTG CGG GAG GAG GTA TCT TTC AGA      6785
Phe Ala Pro Pro Cys Lys Pro Leu Leu Arg Glu Glu Val Ser Phe Arg
    2135                2140                2145

GTA GGA CTC CAC GAG TAC CCG GTG GGG TCG CAA TTA CCT TGC GAG CCC      6833
Val Gly Leu His Glu Tyr Pro Val Gly Ser Gln Leu Pro Cys Glu Pro
2150                2155                2160

GAA CCG GAC GTA GCC GTG TTG ACG TCC ATG CTC ACT GAT CCC TCC CAT      6881
Glu Pro Asp Val Ala Val Leu Thr Ser Met Leu Thr Asp Pro Ser His
2165                2170                2175                2180

ATA ACA GCA GAG GCG GCC GGG AGA AGG TTG GCG AGA GGG TCA CCC CCT      6929
Ile Thr Ala Glu Ala Ala Gly Arg Arg Leu Ala Arg Gly Ser Pro Pro
            2185                2190                2195

TCT ATG GCC AGC TCC TCG GCT AGC CAG CTG TCC GCT CCA TCT CTC AAG      6977
Ser Met Ala Ser Ser Ser Ala Ser Gln Leu Ser Ala Pro Ser Leu Lys
        2200                2205                2210

GCA ACT TGC ACC GCC AAC CAT GAC TCC CCT GAC GCC GAG CTC ATA GAG      7025
Ala Thr Cys Thr Ala Asn His Asp Ser Pro Asp Ala Glu Leu Ile Glu
    2215                2220                2225
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GCT AAC CTC CTG TGG AGG CAG GAG ATG GGC GGC AAC ATC ACC AGG GTT      7073
Ala Asn Leu Leu Trp Arg Gln Glu Met Gly Gly Asn Ile Thr Arg Val
    2230                2235                2240

GAG TCA GAG AAC AAA GTG GTG ATT CTG GAC TCC TTC GAT CCG CTT GTG      7121
Glu Ser Glu Asn Lys Val Val Ile Leu Asp Ser Phe Asp Pro Leu Val
2245                2250                2255                2260

GCA GAG GAG GAT GAG CGG GAG GTC TCC GTA CCC GCA GAA ATT CTG CGG      7169
Ala Glu Glu Asp Glu Arg Glu Val Ser Val Pro Ala Glu Ile Leu Arg
                2265                2270                2275

AAG TCT CGG AGA TTC GCC CCA GCC CTG CCC GTC TGG GCG CGG CCG GAC      7217
Lys Ser Arg Arg Phe Ala Pro Ala Leu Pro Val Trp Ala Arg Pro Asp
            2280                2285                2290

TAC AAC CCC CTG CTA GTA GAG ACG TGG AAA AAG CCT GAC TAC GAA CCA      7265
Tyr Asn Pro Leu Leu Val Glu Thr Trp Lys Lys Pro Asp Tyr Glu Pro
        2295                2300                2305

CCT GTG GTC CAT GGC TGC CCG CTA CCA CCT CCA CGG TCC CCT CCT GTG      7313
Pro Val Val His Gly Cys Pro Leu Pro Pro Pro Arg Ser Pro Pro Val
    2310                2315                2320

CCT CCG CCT CGG AAA AAG CGT ACG GTG GTC CTC ACC GAA TCA ACC CTA      7361
Pro Pro Pro Arg Lys Lys Arg Thr Val Val Leu Thr Glu Ser Thr Leu
2325                2330                2335                2340

CCT ACT GCC TTG GCC GAG CTT GCC ACC AAA AGT TTT GGC AGC TCC TCA      7409
Pro Thr Ala Leu Ala Glu Leu Ala Thr Lys Ser Phe Gly Ser Ser Ser
                2345                2350                2355

ACT TCC GGC ATT ACG GGC GAC AAT ACG ACA ACA TCC TCT GAG CCC GCC      7457
Thr Ser Gly Ile Thr Gly Asp Asn Thr Thr Thr Ser Ser Glu Pro Ala
            2360                2365                2370

CCT TCT GGC TGC CCC CCC GAC TCC GAC GTT GAG TCC TAT TCT TCC ATG      7505
Pro Ser Gly Cys Pro Pro Asp Ser Asp Val Glu Ser Tyr Ser Ser Met
        2375                2380                2385

CCC CCC CTG GAG GGG GAG CCT GGG GAT CCG GAT CTC AGC GAC GGG TCA      7553
Pro Pro Leu Glu Gly Glu Pro Gly Asp Pro Asp Leu Ser Asp Gly Ser
    2390                2395                2400

TGG TCG ACG GTC AGT AGT GGG GCC GAC ACG GAA GAT GTC GTG TGC TGC      7601
Trp Ser Thr Val Ser Ser Gly Ala Asp Thr Glu Asp Val Val Cys Cys
2405                2410                2415                2420

TCA ATG TCT TAT TCC TGG ACA GGC GCA CTC GTC ACC CCG TGC GCT GCG      7649
Ser Met Ser Tyr Ser Trp Thr Gly Ala Leu Val Thr Pro Cys Ala Ala
                2425                2430                2435
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GAG GAA CAA AAA CTG CCC ATC AAC GCA CTG AGC AAC TCG TTG CTA CGC      7697
Glu Glu Gln Lys Leu Pro Ile Asn Ala Leu Ser Asn Ser Leu Leu Arg
            2440                2445                2450

CAT CAC AAT CTG GTG TAT TCC ACC ACT TCA CGC AGT GCT TGC CAA AGG      7745
His His Asn Leu Val Tyr Ser Thr Thr Ser Arg Ser Ala Cys Gln Arg
        2455                2460                2465

AAG AAG AAA GTC ACA TTT GAC AGA CTG CAA GTT CTG GAC AGC CAT TAC      7793
Lys Lys Lys Val Thr Phe Asp Arg Leu Gln Val Leu Asp Ser His Tyr
    2470                2475                2480

CAG GAC GTG CTC AAG GAG GTC AAA GCA GCG GCG TCA AAA GTG AAG GCT      7841
Gln Asp Val Leu Lys Glu Val Lys Ala Ala Ala Ser Lys Val Lys Ala
2485                2490                2495                2500

AAC TTG CTA TCC GTA GAG GAA GCT TGC AGC CTG GCG CCC CCA CAT TCA      7889
Asn Leu Leu Ser Val Glu Glu Ala Cys Ser Leu Ala Pro Pro His Ser
                2505                2510                2515

GCC AAA TCC AAG TTT GGC TAT GGG GCA AAA GAC GTC CGT TGC CAT GCC      7937
Ala Lys Ser Lys Phe Gly Tyr Gly Ala Lys Asp Val Arg Cys His Ala
            2520                2525                2530

AGA AAG GCC GTA GCC CAC ATC AAC TCC GTG TGG AAA GAC CTT CTG GAA      7985
Arg Lys Ala Val Ala His Ile Asn Ser Val Trp Lys Asp Leu Leu Glu
        2535                2540                2545

GAC AGT GTA ACA CCA ATA GAC ACT ACC ATC ATG GCC AAG AAC GAG GTT      8033
Asp Ser Val Thr Pro Ile Asp Thr Thr Ile Met Ala Lys Asn Glu Val
    2550                2555                2560

TTC TGC GTT CAG CCT GAG AAG GGG GGT CGT AAG CCA GCT CGT CTC ATC      8081
Phe Cys Val Gln Pro Glu Lys Gly Gly Arg Lys Pro Ala Arg Leu Ile
2565                2570                2575                2580

GTG TTC CCC GAC CTG GGC GTG CGC GTG TGC GAG AAG ATG GCC CTG TAC      8129
Val Phe Pro Asp Leu Gly Val Arg Val Cys Glu Lys Met Ala Leu Tyr
                2585                2590                2595

GAC GTG GTT AGC AAG CTC CCC TTG GCC GTG ATG GGA AGC TCC TAC GGA      8177
Asp Val Val Ser Lys Leu Pro Leu Ala Val Met Gly Ser Ser Tyr Gly
            2600                2605                2610

TTC CAA TAC TCA CCA GGA CAG CGG GTT GAA TTC CTC GTG CAA GCG TGG      8225
Phe Gln Tyr Ser Pro Gly Gln Arg Val Glu Phe Leu Val Gln Ala Trp
        2615                2620                2625

AAG TCC AAG AAG ACC CCG ATG GGG CTC TCG TAT GAT ACC CGC TGT TTT      8273
Lys Ser Lys Lys Thr Pro Met Gly Leu Ser Tyr Asp Thr Arg Cys Phe
    2630                2635                2640
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GAC TCC ACA GTC ACT GAG AGC GAC ATC CGT ACG GAG GAG GCA ATT TAC      8321
Asp Ser Thr Val Thr Glu Ser Asp Ile Arg Thr Glu Glu Ala Ile Tyr
2645                 2650                 2655                 2660

CAA TGT TGT GAC CTG GAC CCC CAA GCC CGC GTG GCC ATC AAG TCC CTC      8369
Gln Cys Cys Asp Leu Asp Pro Gln Ala Arg Val Ala Ile Lys Ser Leu
                2665                 2670                 2675

ACT GAG AGG CTT TAT GTT GGG GGC CCT CTT ACT AAT TCA AGG GGG GAA      8417
Thr Glu Arg Leu Tyr Val Gly Gly Pro Leu Thr Asn Ser Arg Gly Glu
           2680                 2685                 2690

AAC TGC GGC TAC CGC AGG TGC CGC GCG AGC AGA GTA CTG ACA ACT AGC      8465
Asn Cys Gly Tyr Arg Arg Cys Arg Ala Ser Arg Val Leu Thr Thr Ser
      2695                 2700                 2705

TGT GGT AAC ACC CTC ACT CGC TAC ATC AAG GCC CGG GCA GCC TGT CGA      8513
Cys Gly Asn Thr Leu Thr Arg Tyr Ile Lys Ala Arg Ala Ala Cys Arg
 2710                 2715                 2720

GCC GCA GGG CTC CAG GAC TGC ACC ATG CTC GTG TGT GGC GAC GAC TTA      8561
Ala Ala Gly Leu Gln Asp Cys Thr Met Leu Val Cys Gly Asp Asp Leu
2725                 2730                 2735                 2740

GTC GTT ATC TGT GAA AGT GCG GGG GTC CAG GAG GAC GCG GCG AGC CTG      8609
Val Val Ile Cys Glu Ser Ala Gly Val Gln Glu Asp Ala Ala Ser Leu
                2745                 2750                 2755

AGA GCC TTC ACG GAG GCT ATG ACC AGG TAC TCC GCC CCC CCC GGG GAC      8657
Arg Ala Phe Thr Glu Ala Met Thr Arg Tyr Ser Ala Pro Pro Gly Asp
           2760                 2765                 2770

CCC CCA CAA CCA GAA TAC GAC TTG GAG CTT ATA ACA TCA TGC TCC TCC      8705
Pro Pro Gln Pro Glu Tyr Asp Leu Glu Leu Ile Thr Ser Cys Ser Ser
      2775                 2780                 2785

AAC GTG TCA GTC GCC CAC GAC GGC GCT GGA AAG AGG GTC TAC TAC CTT      8753
Asn Val Ser Val Ala His Asp Gly Ala Gly Lys Arg Val Tyr Tyr Leu
 2790                 2795                 2800

ACC CGT GAC CCT ACA ACC CCC CTC GCG AGA GCC GCG TGG GAG ACA GCA      8801
Thr Arg Asp Pro Thr Thr Pro Leu Ala Arg Ala Ala Trp Glu Thr Ala
2805                 2810                 2815                 2820

AGA CAC ACT CCA GTC AAT TCC TGG CTA GGC AAC ATA ATC ATG TTT GCC      8849
Arg His Thr Pro Val Asn Ser Trp Leu Gly Asn Ile Ile Met Phe Ala
                2825                 2830                 2835

CCC ACA CTG TGG GCG AGG ATG ATA CTG ATG ACC CAC TTC TTT AGC GTC      8897
Pro Thr Leu Trp Ala Arg Met Ile Leu Met Thr His Phe Phe Ser Val
           2840                 2845                 2850
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CTC ATA GCC AGG GAT CAG CTT GAA CAG GCT CTC AAC TGC GAG ATC TAC      8945
Leu Ile Ala Arg Asp Gln Leu Glu Gln Ala Leu Asn Cys Glu Ile Tyr
        2855                2860                2865

GGA GCC TGC TAC TCC ATA GAA CCA CTG GAT CTA CCT CCA ATC ATT CAA      8993
Gly Ala Cys Tyr Ser Ile Glu Pro Leu Asp Leu Pro Pro Ile Ile Gln
    2870                2875                2880

AGA CTC CAT GGC CTC AGC GCA TTT TCA CTC CAC AGT TAC TCT CCA GGT      9041
Arg Leu His Gly Leu Ser Ala Phe Ser Leu His Ser Tyr Ser Pro Gly
2885                2890                2895                2900

GAA ATT AAT AGG GTG GCC GCA TGC CTC AGA AAA CTT GGG GTC CCG CCC      9089
Glu Ile Asn Arg Val Ala Ala Cys Leu Arg Lys Leu Gly Val Pro Pro
                2905                2910                2915

TTG CGA GCT TGG AGA CAC CGG GCC TGG AGC GTC CGC GCT AGG CTT CTG      9137
Leu Arg Ala Trp Arg His Arg Ala Trp Ser Val Arg Ala Arg Leu Leu
            2920                2925                2930

GCC AGA GGA GGC AAG GCT GCC ATA TGT GGC AAG TAC CTC TTC AAC TGG      9185
Ala Arg Gly Gly Lys Ala Ala Ile Cys Gly Lys Tyr Leu Phe Asn Trp
        2935                2940                2945

GCA GTA AGA ACA AAG CTC AAA CTC ACT CCG ATA ACG GCC GCT GGC CGG      9233
Ala Val Arg Thr Lys Leu Lys Leu Thr Pro Ile Thr Ala Ala Gly Arg
    2950                2955                2960

CTG GAC TTG TCC GGC TGG TTC ACG GCT GGC TAC AGC GGG GGA GAC ATT      9281
Leu Asp Leu Ser Gly Trp Phe Thr Ala Gly Tyr Ser Gly Gly Asp Ile
2965                2970                2975                2980

TAT CAC AGC GTG TCT CAT GCC CGG CCC CGC TGG TTC TGG TTT TGC CTA      9329
Tyr His Ser Val Ser His Ala Arg Pro Arg Trp Phe Trp Phe Cys Leu
                2985                2990                2995

CTC CTG CTT GCT GCA GGG GTA GGC ATC TAC CTC CTC CCC AAC CGA          9374
Leu Leu Leu Ala Ala Gly Val Gly Ile Tyr Leu Leu Pro Asn Arg
            3000                3005                3010

TGAAGATTGG GCTAACCACT CCAGGCCAAT AGGCCATTCC CT                      9416
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(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

CAGCCCCCTG ATGGGGGCGA C 21

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

ACTCGCAAGC ACCCTATCA 19

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:495:

CTGTGAGGAA CTACTGTCT 19

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

ATGAGCACGA ATCCTCAAAC CT 22

(2) INFORMATION FOR SEQ ID NO:51:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

GTCCTGCCCT CGGGCC 16

(2) INFORMATION FOR SEQ ID NO:52:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

CGAGGAAGAC TTCCGAGC 18

(2) INFORMATION FOR SEQ ID NO:53:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

ACCCAAATTG CGCGACCTAC 20

(2) INFORMATION FOR SEQ ID NO:54:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

TAAGGTCATC GATACCCT 18

(2) INFORMATION FOR SEQ ID NO:55:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

CAGTTCATCA TCATATCCCA 20

(2) INFORMATION FOR SEQ ID NO:56:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

AGATAGAGAA AGAGCAAC 18

(2) INFORMATION FOR SEQ ID NO:57:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

AGACTTCCGA GCGGTCGCAA 20

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

GACCTGTGCG GGTCTGTC 18

(2) INFORMATION FOR SEQ ID NO:59:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:59:

GGGTCGGCAG CTGGCTAGCC TCTCA 25

(2) INFORMATION FOR SEQ ID NO:60:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:60:

TCCTGGCGGG CATAGCGT 18

(2) INFORMATION FOR SEQ ID NO:61:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:61:

CCCCAGCCCT GGTCAAAATC GGTAA 25

(2) INFORMATION FOR SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:62:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:63:

CTGTCGGTCG TTCCCACCA 19

(2) INFORMATION FOR SEQ ID NO:64:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:64:

CCGCGAAGAG TGTGTGTGGT 20

(2) INFORMATION FOR SEQ ID NO:65:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:65:

CAATGTTCTG GTGGAGGTG 19

(2) INFORMATION FOR SEQ ID NO:66:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 28 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:66:

GCCATTAAGT GGGAGTACGT CGTTCTCC 28

(2) INFORMATION FOR SEQ ID NO:67:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:67:

CGAGGAAGGA TACAAGACC 19

(2) INFORMATION FOR SEQ ID NO:68:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:68:

TGCTTGTGGA TGATGCTACT 20

(2) INFORMATION FOR SEQ ID NO:69:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:69:

CACACGTGCA GTTGCGCT 18

(2) INFORMATION FOR SEQ ID NO:70:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:70:

CTGCTGACCA CTACACAG 18

(2) INFORMATION FOR SEQ ID NO:71:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:71:

GACCAGAGTG GAAGCGCAA 19

(2) INFORMATION FOR SEQ ID NO:72:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:72:

TACCAGAGTC GGGTGTACAG 20

(2) INFORMATION FOR SEQ ID NO:73:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:73:

CTAGGAGGCC CCTTGTCTGC 20

(2) INFORMATION FOR SEQ ID NO:74:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:74:

CTCGGGCCAG CCGATGGA 18

(2) INFORMATION FOR SEQ ID NO:75:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:75:

GGGGACCTCA TGGTTGTCT 19

(2) INFORMATION FOR SEQ ID NO:76:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:76:

CCCGTGGAGT GGCTAAGG 18

(2) INFORMATION FOR SEQ ID NO:77:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:77:

CTCCTCGATG TTGGGATGG 19

(2) INFORMATION FOR SEQ ID NO:78:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:78:

CAGAGCTTCC AGGTGGCTC 19

(2) INFORMATION FOR SEQ ID NO:79:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:79:

CGGGCTCCGT CACTGTG 17

(2) INFORMATION FOR SEQ ID NO:80:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 21 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:80:

GTATTGCAGT CTATCACCGA G 21

(2) INFORMATION FOR SEQ ID NO:81:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:81:

GGCTATACCG GCGACTTCGA 20

(2) INFORMATION FOR SEQ ID NO:82:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:82:

CGTTGAGTGC GGGAGACAG 19

(2) INFORMATION FOR SEQ ID NO:83:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:83:

TCACCATTGA GACAATCACG 20

(2) INFORMATION FOR SEQ ID NO:84:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:84:

GTAAGGAAGG TTCTCCCCAC TC 22

(2) INFORMATION FOR SEQ ID NO:85:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:85:

ATGCCCACTT TCTATCCCAG ACAAAGC 27

(2) INFORMATION FOR SEQ ID NO:86:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:86:

TGCATGTCAT GATGTAT 17

(2) INFORMATION FOR SEQ ID NO:87:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:87:

GGACAAGACG ACCCTGCC 18

(2) INFORMATION FOR SEQ ID NO:88:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:88:

CGTATTGCCT GTCAACAGGC 20

(2) INFORMATION FOR SEQ ID NO:89:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:89:

AGCGCCCACA AAGGCAGTAG 20

(2) INFORMATION FOR SEQ ID NO:90:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:90:

CCTCTTCAAC ATATTGGGG 19

***

(2) INFORMATION FOR SEQ ID NO:91:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:91:

CCAGGAACCG GAGCATGG 18

(2) INFORMATION FOR SEQ ID NO:92:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:92:

ACCAGTGGAT AAGCTCGG 18

(2) INFORMATION FOR SEQ ID NO:93:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:93:

CGTGGTGTAG GCATTAATG 19

(2) INFORMATION FOR SEQ ID NO:94:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:94:

ATGTGGAGTG GGACCTTCC 19

(2) INFORMATION FOR SEQ ID NO:95:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:95:

CTCTGCTGTT ATATGGGAGG 20

(2) INFORMATION FOR SEQ ID NO:96:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:96:

GTTGACGTCC ATGCTCACTG 20

(2) INFORMATION FOR SEQ ID NO:97:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:97:

TTTCCACGTC TCCACTAGCG 20

(2) INFORMATION FOR SEQ ID NO:98:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:98:

GTGAGGACCA CCGTCCGC 18

(2) INFORMATION FOR SEQ ID NO:99:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:99:

TTCCACCTCC AAAGTCCCCT 20

(2) INFORMATION FOR SEQ ID NO:100:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:100:

AGAACTTGCA GTCTGTCAAA TGTGA 25

(2) INFORMATION FOR SEQ ID NO:101:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 34 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:101:

GGAAGAACAG AAACTGCCCA TCAATGCACT AAGC 34

(2) INFORMATION FOR SEQ ID NO:102:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:102:

TGACGCCGCT GCTTTAACCT 20

(2) INFORMATION FOR SEQ ID NO:103:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:103:

TGCAAGCTTC CTCTACGGAT 20

(2) INFORMATION FOR SEQ ID NO:104:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:104:

AGGTTAAAGC AGCGGCGTCA 20

(2) INFORMATION FOR SEQ ID NO:105:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:105:

AGCTTCCCAT CACGGCCAA 19

(2) INFORMATION FOR SEQ ID NO:106:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:106:

GATGGCTTTG TACGACGTG 19

(2) INFORMATION FOR SEQ ID NO:107:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:107:

GCACCTGCGA TAGCCGCAGT 20

(2) INFORMATION FOR SEQ ID NO:108:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:108:

GTCCCTCACC GAGAGGCT 18

(2) INFORMATION FOR SEQ ID NO:109:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:109:

GATTGGAGGT AGATCAAGTG 20

(2) INFORMATION FOR SEQ ID NO:110:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:110:

TACGACTTGG AGCTCATAAC 20

(2) INFORMATION FOR SEQ ID NO:111:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 20 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:111:

AGCAAGACAC ACTCCAGTCA 20

(2) INFORMATION FOR SEQ ID NO:112:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: YES

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:112:

GCCTATTGGC CTGGAGTGGT TAGC 24

(2) INFORMATION FOR SEQ ID NO:113:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:113:

His Val Thr Gly Gly Asn Ala Gly Arg Thr Thr Ala Gly Leu Val Gly
1 5 10 15

Leu Leu Thr Pro Gly Ala Lys Gln Asn Ile
20 25

(2) INFORMATION FOR SEQ ID NO:114:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:114:

His Val Thr Gly Gly Ser Ala Gly His Thr Val Ser Gly Phe Val Ser
1 5 10 15

Leu Leu Ala Pro Gly Ala Lys Gln Asn Val
20 25

(2) INFORMATION FOR SEQ ID NO:115:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:115:

His Val Thr Gly Gly Gln Ala Ala Arg Ala Met Ser Gly Leu Val Ser
1 5 10 15

Leu Phe Thr Pro Gly Ala Lys Gln Asn Ile
20 25

(2) INFORMATION FOR SEQ ID NO:116:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:116:

His Val Thr Gly Gly Arg Val Ala Ser Ser Thr Gln Ser Leu Val Ser
1 5 10 15

Trp Leu Ser Gln Gly Pro Ser Gln Lys Ile
20 25

(2) INFORMATION FOR SEQ ID NO:117:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:117:

His Val Thr Gly Gly Ala Gln Ala Lys Thr Thr Asn Arg Leu Val Ser
1 5 10 15

Met Phe Ala Ser Gly Pro Ser Gln Lys Ile
20 25

(2) INFORMATION FOR SEQ ID NO:118:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:118:

Tyr Thr Ser Gly Gly Ala Ala Ser His Thr Thr Ser Thr Leu Ala Ser
1 5 10 15

Leu Phe Ser Pro Gly Ala Ser Arg Asn Ile
20 25

(2) INFORMATION FOR SEQ ID NO:119:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:119:

His Val Thr Gly Gly Val Gln Gly His Val Thr Ser Thr Leu Thr Ser
1 5 10 15

Leu Phe Arg Pro Gly Ala Ser Gln Lys Ile
20 25

(2) INFORMATION FOR SEQ ID NO:120:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:120:

His Val Thr Gly Gly Ser Ala Gly Arg Thr Thr Ala Gly Leu Val Gly
1 5 10 15

Leu Leu Thr Pro Gly Ala Lys Gln Asn Ile
20 25

(2) INFORMATION FOR SEQ ID NO:121:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 26 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:121:

His Val Thr Gly Gly Ser Ala Gly Arg Ser Val Leu Gly Ile Ala Ser
1 5 10 15

Phe Leu Thr Arg Gly Pro Lys Gln Asn Ile
20 25

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:122:

Val Ala Thr Arg Asp Gly Lys Leu Pro Thr Thr Gln Leu Arg Arg His
1 5 10 15

Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu
20 25 30

(2) INFORMATION FOR SEQ ID NO:123:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:123:

Val Ala Thr Arg Asp Gly Lys Leu Pro Ala Thr Gln Leu Arg Arg His
1 5 10 15

Ile Asp Leu Leu Val Gly Ser Ala Thr Leu Cys Ser Ala Leu
20 25 30

(2) INFORMATION FOR SEQ ID NO:124:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:124:

Leu Ala Ala Arg Asn Ser Ser Ile Pro Thr Thr Thr Ile Arg Arg His
1 5 10 15

Val Asp Leu Leu Val Gly Ala Ala Ala Leu Cys Ser Ala Met
20 25 30

(2) INFORMATION FOR SEQ ID NO:125:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:125:

Leu Ala Ala Arg Asn Val Thr Ile Pro Thr Thr Thr Ile Arg Arg His
1 5 10 15

Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met
20 25 30

(2) INFORMATION FOR SEQ ID NO:126:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:126:

Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Ile Arg Arg His
1 5 10 15

Val Asp Leu Leu Val Gly Ala Ala Ala Phe Cys Ser Ala Met
20 25 30

(2) INFORMATION FOR SEQ ID NO:127:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 30 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:127:

Leu Ala Ala Arg Asn Ala Ser Val Pro Thr Thr Thr Leu Arg Arg His
1 5 10 15

Val Asp Leu Leu Val Gly Thr Ala Ala Phe Cys Ser Ala Met
20 25 30

(2) INFORMATION FOR SEQ ID NO:128:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:128:

Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Ala Gln Gly Trp Gly Pro
1 5 10 15

Ile Ser Tyr Ala Asn Gly Ser Gly Leu Asp Glu
20 25

(2) INFORMATION FOR SEQ ID NO:129:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:129:

Leu Ala Ser Cys Arg Pro Leu Thr Asp Phe Asp Gln Gly Trp Gly Pro
1 5 10 15

Ile Ser Tyr Ala Asn Gly Ser Gly Pro Asp Gln
20 25

(2) INFORMATION FOR SEQ ID NO:130:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:130:

Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Asp Gln Gly Trp Gly Pro
1 5 10 15

Ile Ser His Ala Asn Gly Ser Gly Pro Asp Gln
20 25

(2) INFORMATION FOR SEQ ID NO:131:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:131:

Met Ala Ser Cys Arg Pro Ile Asp Glu Phe Ala Gln Gly Trp Gly Pro
1 5 10 15

Ile Thr His Asp Met Pro Glu Ser Ser Asp Gln
20 25

(2) INFORMATION FOR SEQ ID NO:132:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:132:

Met Ala Gln Cys Arg Thr Ile Asp Lys Phe Asp Gln Gly Trp Gly Pro
1 5 10 15

Ile Thr Tyr Ala Glu Ser Ser Arg Ser Asp Gln
20 25

(2) INFORMATION FOR SEQ ID NO:122:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:133:

Met Ala Ser Cys Arg Pro Ile Gln Trp Phe Ala Gln Gly Trp Gly Pro
1 5 10 15

Ile Thr Tyr Thr Glu Pro Asp Ser Pro Asp Gln
20 25

(2) INFORMATION FOR SEQ ID NO:134:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 27 amino acids
(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:134:

Leu Ala Ser Cys Arg Arg Leu Thr Asp Phe Asp Gln Gly Trp Gly Pro
1 5 10 15

Ile Ser Tyr Ala Asn Gly Ser Gly Pro Asp Glu
20 25

(2) INFORMATION FOR SEQ ID NO:135:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:135:

Ser Thr Ser Gly Ile Thr Gly Asp Asn Thr Thr Thr Ser Ser Glu Pro
1 5 10 15

Ala Pro Ser Gly Cys Pro Pro Asp
20

(2) INFORMATION FOR SEQ ID NO:136:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:136:

Gly Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Gly Pro Pro Asp Gln
1 5 10 15

Ala Ser Asp Asp Gly Asp Lys Gly
20

(2) INFORMATION FOR SEQ ID NO:137:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 amino acids
(B) TYPE: amino acid
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:137:

Glu Ser Ser Ala Val Asp Ser Gly Thr Ala Thr Ala Leu Pro Asp Gln
1 5 10 15

Ala Ser Asp Asp Gly Asp Lys Gly
20

What Is Claimed Is:

1. A DNA sequence encoding the genome of a non-A, non-B hepatitis virus (NANBV) belonging to the Hutch subgroup, said DNA sequence being selected from the group of the following DNA sequences:

(a) the Hutch c59 DNA sequence shown in SEQ ID NO:46;

(b) a DNA sequence encoding the same polyprotein as the DNA sequence (a) but which differs from said DNA sequence (a) as a result of the degeneration of the genetic code;

(c) a DNA sequence which hybridizes to said DNA sequence (a) or (b) and represents a mutant or variant of the NANBV Hutch c59 strain displaying essentially the same specific immunological properties; and

(d) a DNA sequence which hybridizes to said DNA sequence (a) or (b) and represents a NANBV strain having the immunological properties of the Hutch subgroup.

2. A DNA sequence having a length of about 10 to 200 nucleotides that corresponds to a portion of the DNA sequence of claim 1, said DNA sequence having at least one nucleotide difference in sequence when compared to the nucleotide sequence of a strain of NANBV selected from the group consisting of HCV-1, HCV-BK, HCV-J, HC-J1, HC-J4, HCV-JH and HCV-Hh, wherein said nucleotide difference represents a silent mutation or a mutation causing a difference in at least one amino acid.

3. A DNA sequence encoding a variable region of the NANBV genome or a portion thereof, said region or portion thereof having an amino acid sequence, the SEQ

ID NO and corresponding residues of which are shown in parenthesis, selected from the group consisting of:

(a) the V variable region:
-HVTGGNAGRTTAGLVGLLTPGAKQNI-
(46 : 386-411);

(b) a part of V:
-NAGRTTAGLVGLLT-
(46 : 391-404);

(c) the V_1 variable region:
-VATRDGKLPTTQLRRHIDLLVGSATL
GSAL- (46 : 246-275);

(d) a part of V_1:
-VATRDGKLPTT-
(46 : 246-256);

(e) the V_2 variable region:
-LASCRRLTDFAQGWGPISYANGSGLDE-
(46 : 456-482);

(f) a part of V_2:
-RLTDFA-
(46 : 461-466)

(g) a part of V_2:
-SYANGSGLDE-
(46 : 473-482); and

(h) the V_3 variable region
-STSGITGDNTTTSSEPAPSGCPPD-
(46 : 2356-2379).

4. A DNA sequence derived from a NANBV genome and encoding a variable region or a portion thereof corresponding to the variable region or portion thereof encoded by any of the DNA sequences characterized in claim 3.

5. A DNA sequence having a length of about 18 to 200 nucleotides comprising a DNA sequence of claim 3 or 4.

6. A DNA sequence according to claim 3 or 5 corresponding to a sequence shown in SEQ ID NO:46.

7. A DNA sequence that encodes the NANBV structural capsid protein having an amino acid sequence contained in SEQ ID NO:1 from residue 1 to 120 or that encodes an immunologically active part of said protein.

8. A DNA sequence that hybridizes to the DNA sequence of claim 7 and that encodes a NANBV structural capsid protein or an immunologically active part thereof.

9. A DNA sequence encoding a part of the NANBV structural capsid protein having an amino acid sequence contained in SEQ ID NO:1 from residue 1 to residue 20.

10. A DNA sequence encoding a part of the NANBV structural capsid protein having an amino acid sequence contained in SEQ ID NO:1 from residue 1 to residue 74.

11. A DNA sequence encoding a part of the NANBV structural capsid protein having an amino acid sequence contained in SEQ ID NO:1 from residue 21 to residue 40.

12. A DNA sequence encoding a part of the NANBV structural capsid protein having an amino acid sequence contained in SEQ ID NO:1 from residue 2 to residue 40.

13. A DNA sequence encoding a part of the NANBV structural capsid protein having an amino acid sequence contained in SEQ ID NO:1 from residue 69 to residue 120.

14. A DNA sequence encoding a part of the NANBV structural capsid protein having an amino acid sequence contained in SEQ ID NO:1 from residue 121 to residue 326 or that encodes an immunologically active part of said protein.

15. A DNA sequence that hybridizes to the DNA sequence of claim 14 and that encodes a NANBV structural envelope protein or an immunologically active part thereof.

16. A DNA sequence encoding a part of the NANBV structural envelope protein having an amino acid sequence contained in SEQ ID NO:1 from residue 121 to 176.

17. A DNA sequence that encodes the amino acid sequence contained in SEQ ID NO:1 from residue 1 to residue 326 or that encodes an immunologically active part of said amino acid sequence.

18. A DNA sequence comprising a DNA sequence of any one of claims 1 to 17.

19. A recombinant DNA molecule comprising a vector operatively linked to a DNA sequence according to any one of claims 1 to 18.

20. The recombinant DNA molecule of claim 19 wherein said vector is an expression vector, said molecule is capable of expressing said protein in a compatible host, and said NANBV structural protein has an amino acid residue sequence shown in SEQ ID NO:2 from residue 1 to residue 315.

21. The recombinant DNA molecule of claim 19 wherein said vector is an expression vector, said molecule is capable of expressing said protein in a compatible host, and said NANBV structural protein has an amino acid residue sequence contained in SEQ ID NO:3 from residue 1 to residue 252.

22. The recombinant DNA molecule of claim 19 wherein said vector is an expression vector, said molecule is capable of expressing said protein in a compatible host, and said NANBV structural protein has an amino acid residue sequence contained in SEQ ID NO:4 from residue 1 to residue 252.

23. The recombinant DNA molecule of claim 19 wherein said vector is an expression vector and said molecule is capable of expressing said protein in a compatible host, and said NANBV structural protein has an amino acid residue sequence contained in SEQ ID NO:6 from residue 1 to residue 271.

24. A transformed host cell containing a DNA sequence of any one of claims 1 to 18 or a recombinant DNA molecule according to any one of claims 19 to 23.

25. A polypeptide or peptide encoded by a DNA sequence of any one of claims 1 to 18 or a recombinant DNA molecule of any one of claims 19 to 23.

26. The polypeptide or peptide of claim 25 having a length from about 7 to about 200 amino acid residues.

27. The polypeptide or peptide of claim 25 which is a NANBV structural protein having a length of at least 20 amino acids.

28. A composition comprising at least one polypeptide or peptide according to any one of claims 25 to 27.

29. An antibody that immunoreacts with a polypeptide or peptide according to any one of claims 25 to 27, but does not immunoreact with NANBV isolates HCV-1, HCV-BK, HCV-J, HC-J1, HC-J4, HCV-JH or HCV-Hh.

30. An antibody that immunoreacts with the Hutch c59 isolate of NANBV or a part thereof, but does not immunoreact with NANBV isolates HCV-1, HCV-BK, HCV-J, HC-J1, HC-J4, HCV-JH or HCV-Hh.

31. A diagnostic kit for assaying a body fluid sample for the presence of antibodies against NANBV structural antigens comprising, in an amount sufficient to perform at least one assay, at least one polypeptide or peptide according to any one of claims 25 to 27.

32. The diagnostic kit according to claim 31 wherein said polypeptide or peptide is affixed to a solid matrix.

33. A diagnostic kit for assaying a body fluid sample for the presence of NANBV structural antigens comprising, in an amount sufficient to perform at least one assay, an anti-NANBV structural protein antibody that:

(i) immunoreacts with (a) the Hutch c59 isolate of NANBV, (b) a polypeptide or peptide according to any one of claims 25 to 27;

(ii) but does not immunoreact with (c) NANBV isolates HCV-1, HCV-BK, HCV-J, HC-J1, HC-J4, HCV-JH or HCV-Hh, or (d) the C-100 antigen.

34. The diagnostic kit of claim 33 wherein said antibody is affixed to a solid matrix.

35. A method of assaying a body fluid sample for the presence of antibodies against a NANBV structural antigen, which method comprises:

(a) forming an aqueous immunoreaction admixture by admixing said body sample with a polypeptide or peptide of any one of claims 25 to 27;

(b) maintaining said aqueous immunoreaction admixture for a time period sufficient for any of said antibodies present to immunoreact with said polypeptide or peptide to form an immunoreaction product; and

(c) detecting the presence of any of said immunoreaction product formed and thereby the presence of said antibodies.

36. The method of claim 35 wherein said polypeptide or peptide is affixed to a solid matrix.

37. The method of claim 36 wherein said detecting in step (c) comprises the steps of:

(i) admixing said immunoreaction product formed in step (c) with a labeled specific binding agent to form a labeling admixture, said labeled specific binding agent comprising a specific binding agent and a label;

(ii) maintaining said labeling admixture for a period sufficient for any of said immunoreaction product present to bind with said labeled specific binding agent to form a labeled product; and

(iii) detecting the presence of any of said labeled product formed, and thereby the presence of said immunoreaction product.

38. The method of claim 37 wherein said specific binding agent is selected from the group consisting of Protein A and at least one of the antibodies anti-human IgG and anti-human IgM.

39. The method of claim 37 wherein said label is selected from the group consisting of lanthanide chelate, biotin, enzyme and radioactive isotope.

40. A method of assaying a body sample for the presence of NANBV polynucleic acids which method comprises:

(a) forming an aqueous hybridization admixture by admixing said body sample with a polynucleotide or oligonucleotide having a DNA sequence according to any one of claims 1 to 18;

(b) maintaining said aqueous hybridization admixture for a time period and under hybridizing conditions sufficient for any of said NANBV polynucleic acids present to hybridize with said polynucleotide or oligonucleotide to form a hybridization product; and

(c) detecting the presence of any of said hybridization product formed and thereby the presence of said NANBV polynucleic acids.

41. A method of assaying a body fluid sample for the presence of NANBV structural antigens, which method comprises reacting said sample with an antibody according to claim 29 or 30.

42. An inoculum comprising an immunologically effective amount of a polypeptide or peptide according to any one of claims 25 to 27, said polypeptide or peptide being either alone or linked to an antigenic carrier and dispersed in a pharmaceutically acceptable excipient.

43. A vaccine comprising an immunologically effective amount of a polypeptide or peptide according to any one of claims 25 to 27, said polypeptide or peptide being either alone or linked to an antigenic carrier and dispersed in a pharmaceutically acceptable excipient.

44. A method of producing a NANBV structural protein comprising:

(a) initiating a culture comprising a nutrient medium containing transformed host cells according to claim 24;

(b) maintaining the culture for a time period sufficient for the transformed host cells to express NANBV structural protein; and

(c) recovering the NANBV structural protein from the culture.

45. A method for inducing antibody production in a mammal, said antibody being immunoreactive with NANBV, comprising (a) administering an inoculum according to claim 42 or a vaccine according to claim 43 to said mammal, and (b) maintaining the mammal for a time period sufficient for said mammal to respond immunologically and produce anti-NANBV antibody.

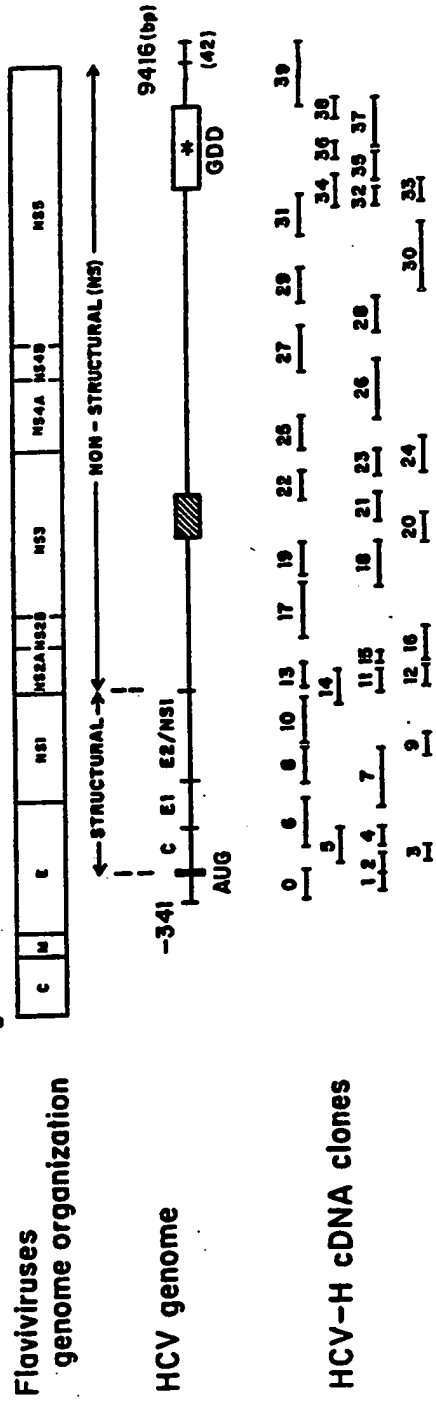
Flaviviruses
genome organization
HCV genome
HCV-H cDNA clones
= NS3 dengue type 2
= replicase CARMV
5'
3'
C
M
E
NS1
NS2A
NS2B
NS3
NS4A
NS4B
NS5
NON-STRUCTURAL (NS)
STRUCTURAL
C
E1
E2/NS1
AUG
-341
9416(bp)
(42)
GDD

FIGURE 1

INTERNATIONAL SEARCH REPORT

International Application No. PCT/US91/06037

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

U.S. CL.:536/27; 530/350, 387; 435/5; 424/89
IPC(5):C07H 15/12; C07K 3/00; C12Q 1/70; A61K 39/12

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
U.S.	536/27; 530/350, 387; 435/5; 424/89

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

STIC Sequence Search
APS

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	Gene. Volume 91, issued 1990, K. Takeuchi et al.. "Hepatitis C viral cDNA isolated from a healthy carrier donor implicated in post-transfusion non-A, non-B hepatitis," pages 287-291, see entire document.	1,3-6
X Y	EP, A. 0.318,216 (Houghton et al.) 31 May 1989. see figures and claims.	16 3-15,17-45
X Y,P	EP. A. 0,388,232 (Houghton et al.) 19 September 1990. see figures and claims.	16 1-15,17-45

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
07 January 1992	24 JAN 1992
International Searching Authority	**Signature of Authorized Officer**
ISA/US	Donna C. Wortman ebw

Form PCT/ISA/210 (second sheet) (Rev.11-87)

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers . because they relate to subject matter [12] not required to be searched by this Authority, namely:

2. ☐ Claim numbers ... . . . , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out [13], specifically:

3. ☐ Claim numbers_____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

See attached sheet.

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. Telephone practice

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

Attachment to PCT/IPEA/210

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, Claims 1-24 and 40, drawn to DNA sequences, recombinant DNA, transformed host cell, and first method of use, classified in Class 536, subclass 27;

Claim 1 is generic, the first species is recited in claim 2 (a), and the following additional species are present:

species 2, as recited in Claim 2 (b)
species 3, as recited in Claim 3 (c)
species 4, as recited in Claim 3 (d)
species 5, as recited in Claim 3 (e)
species 6, as recited in Claim 3 (f)
species 7, as recited in Claim 3 (g)
species 8, as recited in Claim 3 (h)
species 9, as recited in Claim 7
species 10, as recited in Claim 9
species 11, as recited in Claim 10
species 12, as recited in Claim 11
species 13, as recited in Claim 12
species 14, as recited in Claim 13
species 15, as recited in Claim 14
species 16, as recited in Claim 16
species 17, as recited in Claim 17
species 18, as recited in Claim 20
species 19, as recited in Claim 21
species 20, as recited in Claim 22
species 21, as recited in Claim 23

Group II, claims 25-28, 31, and 32, drawn to polypeptides, classified in class 530, subclass 350.

Group III, claims 29, 30, 33, 34, 41, drawn to antibody compositions, classified in Class 530, subclass 387, and Class 435, subclass 5.

Group IV, claims 35-39, drawn to method using polypeptides, classified in Class 435, subclass 5.

Group V, claim 42, 43, 45, drawn to a vaccine, classified in class 424, subclass 89.

Group VI, Claim 44, drawn to a method of making a protein, classified in Class 530, subclass 350.

The claims of these six groups are drawn to distinct inventions which are not linked so as to form a single general inventive concept. PCT Rule 13.1 and 13.2 do not provide for multiple products and methods.

Attachment to PCT Telephone Memorandum for
Lack of Unity of Invention

Group I, Claims 1-24 and 40, drawn to DNA sequences, recombinant DNA, transformed host cell, and first method of use, classified in Class 536, subclass 27;

Claim 1 is generic, the first species is recited in claim 3 (a), and the following additional species are present:

species 2, as recited in Claim 3 (b)
species 3, as recited in Claim 3 (c)
species 4, as recited in Claim 3 (d)
species 5, as recited in Claim 3 (e)
species 6, as recited in Claim 3 (f)
species 7, as recited in Claim 3 (g)
species 8, as recited in Claim 3 (h)
species 9, as recited in Claim 7
species 10, as recited in Claim 9
species 11, as recited in Claim 10
species 12, as recited in Claim 11
species 13, as recited in Claim 12
species 14, as recited in Claim 13
species 15, as recited in Claim 14
species 16, as recited in Claim 16
species 17, as recited in Claim 17
species 18, as recited in Claim 20
species 19, as recited in Claim 21
species 20, as recited in Claim 22
species 21, as recited in Claim 23

Group II, claims 25-28, 31, and 32, drawn to polypeptides, classified in class 530, subclass 350.

Group III, claims 29, 30, 33, 34, 41, drawn to antibody compositions, classified in Class 530, subclass 387, and Class 435, subclass 5.

Group IV, claims 35-39, drawn to method using polypeptides, classified in Class 435, subclass 5.

Group V, claim 42, 43, 45, drawn to a vaccine, classified in class 424, subclass 89.

Group VI, Claim 44, drawn to a method of making a protein, classified in Class 530, subclass 350.

The claims of these six groups are drawn to distinct inventions which are not linked so as to form a single general inventive concept. PCT Rule 13.1 and 13.2 do not provide for multiple products and methods.